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MISSOURI

BOTANICAL GARDEN

TWENTY-THIRD ANNUAL REPORT

ST. LOUIS, MO.
PUBLISHED BY THE BOARD OF TRUSTEES
1912

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OF THE MISSOURI BOTANICAL GARDEN

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[1] Elected President of the Academy of Science of St. Louis, January 17, 1912, to succeed William Trelease, who held that office for three years.

[2] Elected President of the School Board November 13, 1912, to succeed Rhodes E. Cave, who held that office for one year.

GOVERNMENT BOTANICAL GARDENS
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STAFF
OF THE MISSOURI BOTANICAL GARDEN

January 1 to May 1, 1912.

Director
WILLIAM TRELEASE.

GEORGE T. MOORE,
Plant Physiologist.

HERMANN VON SCHRENK,
Plant Pathologist.[1]

Research Assistant.

CORA J. HOGAN,
Curator of Library.

MOSES CRAIG,
Curator of Herbarium.

HENRY C. IRISH,
Superintendent.

JAMES GURNEY,
Head Gardener.[2]

JOHN BANNES,
Foreman.[2]

ADOLPH JAENICKE,
Foreman.

May 1 to December 31, 1912.

Director
GEORGE T. MOORE.

BENJAMIN MINGE DUGGAR,
Plant Physiologist.

HERMANN VON SCHRENK,
Plant Pathologist.[1]

GEORGE R. HILL, JR.,
Research Assistant.

CORA J. HOGAN,
Curator of Library.

MOSES CRAIG,
Curator of Herbarium.

JACOB R. SCHRAMM,
Assistant to the Director.[4]

ADOLPH JAENICKE,
Head Gardener.

CHARLES H. THOMPSON,
Assistant Botanist.

HENRY C. IRISH,
Superintendent.[3]

JAMES GURNEY,
Head Gardener.[2]

[1] *Honorary.*
[2] *Emeritus.*
[3] Resigned July 1, 1912.
[4] Appointed July 1, 1912.

PREFACE

The twenty-third annual report of the Missouri Botanical Garden comprises the report of the officers of the Board, the twenty-third annual report of the former Director and seven scientific papers, the result, for the most part, of work done in the Graduate Laboratory at the Garden.

The twenty-second report was issued on February 14, 1912, which marks the date of publication of the scientific papers contained.

The reports are sent to scientific institutions and a limited number of individuals, in exchange for publications or desirable material for the various Garden collections. They may be purchased at approximately the cost of publication from the Missouri Botanical Garden, Messrs. R. Friedländer & Sohn, Berlin, or William Wesley & Son, London.

George T. Moore.

St. Louis, Mo., December 1, 1912.

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REPORTS FOR THE YEAR 1911.

REPORT OF THE OFFICERS OF THE BOARD.

SUBMITTED TO THE TRUSTEES, JANUARY 10, 1912.

To the Board of Trustees of the Missouri Botanical Garden:

We submit for your consideration the financial results for the year ending December 31, 1911.

The earnings from rentals exceeded those of the year 1910 by $6,321.78, but did not meet our expectations for the reason that several vacancies in business property existed throughout the entire year and still exist. Our loss in revenue for that reason amounted to $11,679.00, and out of the total earnings $182.00 was charged off as uncollectible—about one-seventh of one per cent.

One of our buildings, on account of age, was found to be in a dangerous condition, and we were compelled to rebuild it at a cost of $6,585.67, which, with necessary repairs to others, caused an unusually large expenditure.

Without counting any rentals from buildings now vacant, we can safely expect the rentals for the coming year to exceed those of 1910 by at least $11,000.00.

During the past year we succeeded in acquiring, by purchase, the lot owned by the Mount Calvary Episcopal Church, which has prevented the extension of Lafayette Avenue through the Board's property lying just west of Grand Avenue, and by this purchase we have been able to extend Lafayette Avenue west practically on the same line for a distance of about 5,500 feet, and it is now fully improved for a distance of 2,225 feet, and some houses have already been erected.

The improvements begun upon this tract, known as Shaw's Lafayette Avenue Addition, last year, have been carried from Spring to Grand Avenues, and are finished at an additional cost of $39,881.52, thus enabling us, without further expense, to dispose of about 10,000 front feet of very desirable residence property.

We have still left in this tract about 20,000 front feet, some of which is partially improved, which we can fully improve at our leisure, as the outside streets are all made, when there is sufficient demand for it, at approximately an additional cost of $150,000.00, which sum, with other amounts, will come back to us by increased values as the property is sold.

We have expended during the year the following amounts for street improvements:

Lafayette Avenue Addition	Sundries	$39,881 52
Kingshighway	Roadway	9,427 56
Arsenal Street	Roadway	6,971 85
McRee Avenue	Roadway	18,743 06
Vandeventer Avenue	Sidewalks	1,621 24
Total		$76,645 23

During the past year we have disposed of vacant property, as follows:

Lafayette Avenue Addition	2,743 Front feet	$107,816 75
Flora Boulevard Addition	320 Front feet	22,510 00
Total		$130,326 75

We have, in addition, signed contracts for the sale of additional property, which will shortly be closed, for 1,054 front feet, amounting to $70,000.00.

In our opinion we have almost reached a point where no further street improvements can be forced upon us by ordinance, with the exception of the following, which we may be compelled to make this year:

Shaw Avenue, west of Tower Grove	$14,000 00
Thurman Avenue, north of Shaw	10,000 00
Sidewalks, sundries	5,000 00
	$29,000 00

The Board has for years felt the necessity for additional plant house space at the Garden, but have been prevented from making any extensive improvements on account of the seemingly never-ending demands for street improvements, the expenditures for which, during the past fifteen years, have reached the sum of $401,000.00, but they now feel that the surplus of receipts over expenditures during the past twenty years, amounting to $179.399.71, which has been used for street improvements, will soon be reimbursed by sales of property, thus making it possible to make an expenditure for an additional plant house.

In view of the above facts, we recently entered into a contract with an architect and builder of plant houses of national reputation, to erect during the summer a very large and completely equipped plant house and a steam plant, equal to any in this country, at a cost of about $135,000.00, which will be finished and ready for occupancy early in October, 1912.

The Library and Herbarium collections have received additions by purchase and donations during the year, valued at the following sums, which have been credited to stock account:

Library	$4,828 63
Herbarium	2,489 10

The Annual Bequests provided for in Mr. Shaw's will, with the exception of the Trustees' Annual Banquet, have been met by the expenditure of $1,376.80, but the full amounts authorized, $2,100.00, have been charged against the year's income to be used at some future time.

After charging against the income all of the expenses, except $76,645.23, expended for street improvements, we are able to carry forward as a surplus for the year, the sum of $7,860.02.

RECEIPTS.

Rentals	$141,541 07	
Interest and dividends	4,157 34	
Garden Handbook sales	79 65	
Publication sales	32 37	
Garden sales	22 43	
Total income receipts		$145,832 86
Sales of real estate under decree	92,457 59	
Bills receivable, account of real estate	12,415 93	
Shaw School of Botany, rentals	4,225 00	109,098 52
Total receipts		$254,931 38
Cash on hand December 31, 1910		43,726 30
		$298,657 68

DISBURSEMENTS.

Garden Account,			
Labor pay-roll	$24,883 86		
Students' pay-roll	1,706 76		
Office assistance	1,125 00	$27,715 62	
Fuel		2,374 99	
Water		641 00	
Repairs and supplies		3,155 21	
Stable and implements		585 44	
Plants and seeds		1,084 86	
Total for care of Garden			$35,557 12
Herbarium Account,			
Salaries		1,073 55	
Fuel		273 39	
Current expense and additions		1,302 98	2,649 92
Library Account,			
Salaries		2,424 17	
Fuel		364 52	
Current expense and additions		2,585 38	5,374 07
Garden Office Account,			
Salaries		5,485 25	
Fuel		250 62	
Current expense		670 53	6,406 40
Research and Instruction,			
Salaries		8,876 92	
Fuel		250 64	
Current expense and instruments		1,884 93	11,012 49
Total Garden maintenance			$61,000 00
Carried forward			$61,000 00

Amount brought forward . .		$61,000 00
Garden Improvement,		
Furnishing library and herbarium . .	450 00	
Plant house improvements	985 38	
Garden renovation	934 78	
Stone fence and steps	1,678 75	
Sewer extension	748 25	
Traveling expenses	275 00	5,072 16
Total amount expended on Garden .		$66,072 16
Publication Account,		
Twenty-second Annual Report, account of	506 06	
Thirteenth Annual Report, reprint . .	500 00	
Garden Handbook	494 59	1,500 65
Property Account,		$67,572 81
State, school and city taxes	$35,366 30	
Streets, sidewalks and sewers . . .	73,215 12	
Insurance	4,639 76	
Repairs	6,549 30	
Improvements	6,986 79	126,757 27
Bequests,		
Annual Flower Sermon	200 00	
Annual Flower Show	436 00	
Annual Gardener's Banquet	740 80	1,376 80
Sundries,		
Office expenses	6,681 27	
Legal and professional services . . .	541 05	
Commissions	5,740 20	12,962 52
Shaw School of Botany, rentals	4,875 00	4,875 00
Investments	40,000 00	40,000 00
Mount Calvary Episcopal Church, Property at Grand and Lafayette Avenues	30,000 00	30,000 00
Total Disbursements		$283,544 40
Cash balance December 31, 1911 . .		15,113 28
		$298,657 68

Respectfully submitted,

EDWARDS WHITAKER, President.

Attest:

A. D. CUNNINGHAM, Secretary.

TWENTY-THIRD ANNUAL REPORT OF THE DIRECTOR.

SUBMITTED TO THE TRUSTEES JANUARY 10, 1912.

To the Board of Trustees of the Missouri Botanical Garden:

The following report on the Missouri Botanical Garden and the School of Botany therewith connected is respectfully submitted, in compliance with your rules.

GARDENING.

This year, under authorization of the Board, the central section of the Garden, heretofore, as during Mr. Shaw's lifetime, largely maintained in the form of narrow beds separated by gravel paths and hedges of various kinds, has been reconstructed in accordance with the plans of the landscape architects of the Board; the observatory marking the center of a circular plot subdivided into eight sectors, enclosed by low hedges, separated by radiating walks, and given over to flower beds parted by turf. This has made possible the cultivation of a considerably increased number of flowering plants, selected with a view to securing persistent color throughout the season. Though the area heretofore allotted to formal decorative planting has not been enlarged, the customary variation in material used has been supplemented by an increased display of flowering herbaceous plants flanking the shrubbery backgrounds; under the efficient care of the Foreman, Mr. Jaenicke, notwithstanding a season very unfavorable in some respects, the color effect has been brighter than usual.

As in past years, the sunken garden was occupied in early spring by tulips, of which 28,000 bulbs were used, representing 225 varieties. Later the same ground was used for

bedding plants, set off to advantage by a massed cluster of palms in the center. The number of plants used was 45,000,—about 7,000 more than in 1910.

For the first time since special effort has been made to grow chrysanthemums, the Garden this fall failed to offer to the public the customary display. The usual provision for this event was made, and a collection of 503 varieties, represented by 2,700 well-grown plants, brought into flower and arranged under canvas ready to be exhibited, when a heavy gale shredded the tent on the night of November 11, and a fall of over 70° in temperature before morning entirely ruined the collection, except that material was preserved for propagation next year,—when the plants will be staged under glass.

As for two years preceding, Oenotheras have been grown in large numbers (about 10,000), for the studies of Dr. Gates, and small, but important, cultures have been made of variegated plants, cranberries, chrysanthemums, and caladiums, in connection with work being conducted by Research Fellows under the direction of Professor Moore.

THE PLANT COLLECTION.

The following table indicates the number of additions made during the year to the living collections:

	Accessions	Plants or Packets	Value
Bought	42	11,838	$1,079 46
Presented	231	8,636	459 55
Collected by employees			
In the Garden	1	2,240	403 20
Elsewhere	20	287	77 05
	294	23,001	$2,019 26
Cuttings raised	1	27,737	1,386 85
Seedlings raised	1	36,700	2,569 00
	296	87,438	$5,975 11

The accessions added 1,635 species or varieties to the living collections; 1,041 were lost or discarded. The net

gain for the year is 594, bringing the total to 12,668, as compared with the 12,074 grown in 1910.[1]

DISTRIBUTION OF PLANTS AND SEEDS.

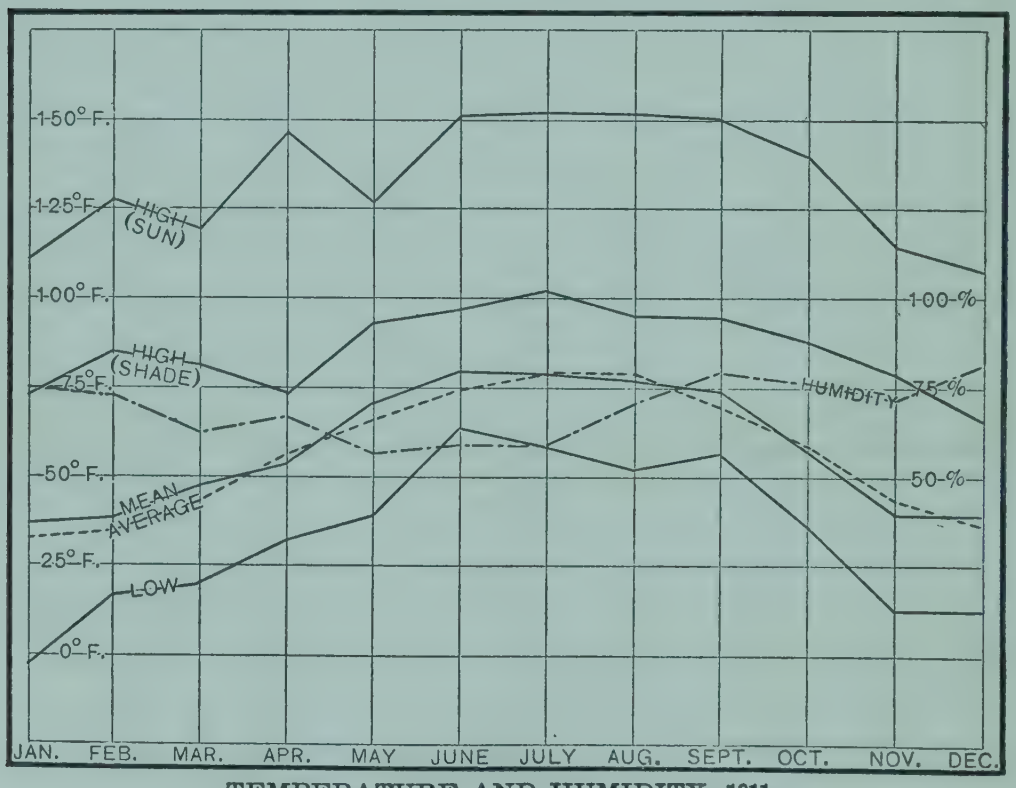
The exchange seed list of 1910 numbered 2,673 species and varieties. From it correspondents of the Garden have selected 9,884 packets of seeds, valued at $494.20; and 1,272 living plants, valued at $123.40, have been sent to exchanging institutions,—the exchange return, as indicated in the table, being 8,636 plants or seed packets, valued at $459.55.

Surplus plants to the number of 1,425, estimated at $133.75, have been given to schools and colleges for educational use; 1,592 surplus plants were given to charities.

THE WEATHER.

Few seasons have been as trying to gardeners as the one just closed. Temperature and precipitation fluctuated far

DIAGRAM A.

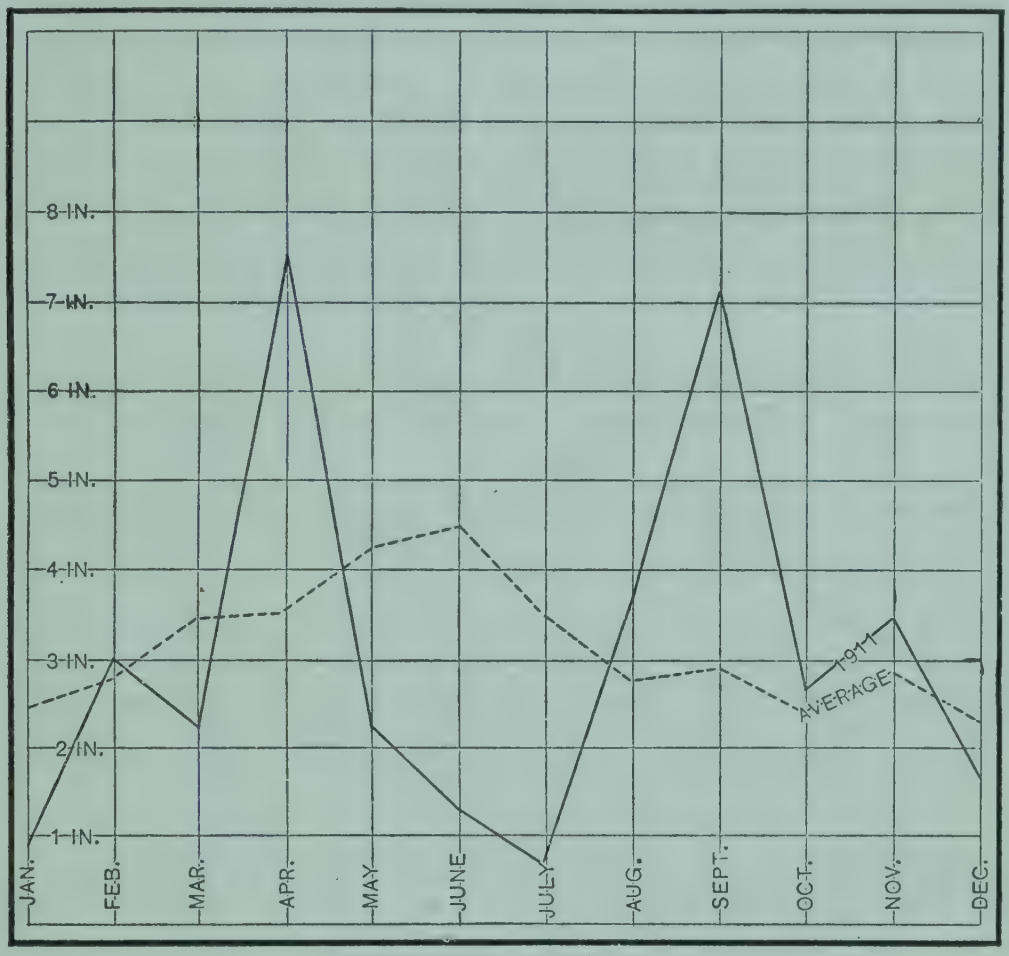


TEMPERATURE AND HUMIDITY, 1911.

[1] Rept. Mo. Bot. Gard. **23** : 16.

more than usual and worked harm at both extremes. The appended diagrams (A, B) show the weather curves for the year in contrast with the average for the earlier period covered by the reports of the local Weather Bureau, from which the diagrams have been prepared.

DIAGRAM B.



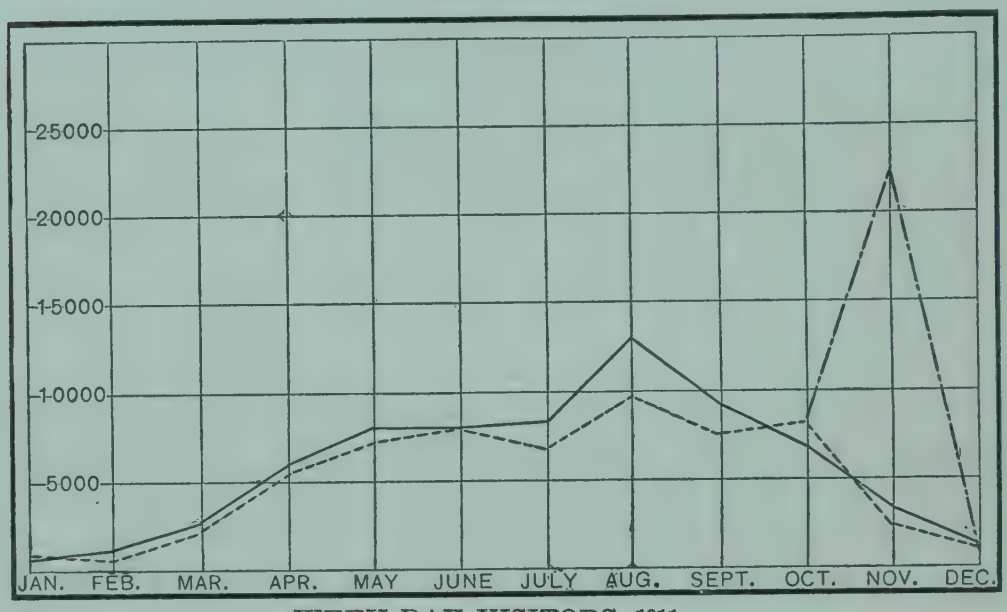
PRECIPITATION, 1911.

VISITORS.

The total number of visitors to the Garden in 1911 was 91,243. Of these, 7,794 were recorded on the first Sunday afternoon in June and 14,760 on the corresponding Sunday in September, when, under provision of Mr. Shaw's will, the Garden was open to the public. The remaining 68,689 visitors were counted on week-days.

Since 1903, when the total reached 79,039, the number of visitors has never been so small as in the year just closed. Except for February, October and December, the record for each month is smaller than in 1910; the loss of the

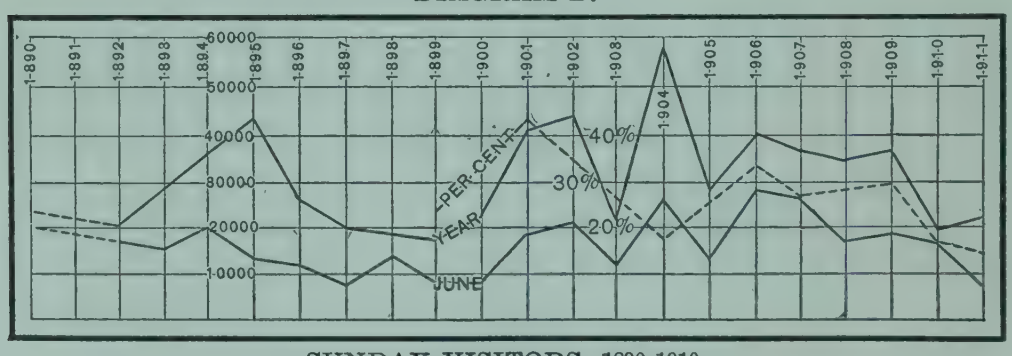
DIAGRAM C.



WEEK-DAY VISITORS, 1911.

chrysanthemums reduced the number of November visitors by about 19,000, judging from the November average for the preceding seven years. Notwithstanding this, the number recorded for 1911 equals the earlier average (excluding

DIAGRAM D.



SUNDAY VISITORS, 1890-1910.

the Exposition year) for June and surpasses it for every other month, except January, October and November; and it exceeds the November average for the period ending with

1903. An interesting fact shown by the appended diagram (C), presenting the seasonal distribution of week-day visitors, is that this year, for the first time, a characteristic falling off in the number of visitors for July does not appear.

IMPROVEMENTS.

One of the most important betterments of the Garden yet made has become possible this year through the co-operation of the City Sewer Department, the Commissioners of Tower Grove Park, the Trustees of the Garden and the owners of largely improved property lying to the southwest of the grounds, by which a new sewer district has been established and a connection made across the Garden pasture with the city sewer system. As a result of this, all of the sewage from this residence property, the Park, and the adjoining Garden has been diverted from the upper part of the brook that crosses the pasture and arboretum. At the same time, all of the foul water from the center of the Garden has been diverted from the lower brook into the Russell Avenue sewer, so that the brook will carry only storm water in the future,—a result long desired, but impossible without such provision as has now been made by the City.

The first large undertaking in development of the Garden since the North American section was graded and planted ten years ago has been inaugurated this year by arranging for a large modern plant house conformed to the plans of the landscape architects of the Board. After a thorough investigation, supplemented by the study of competitive designs submitted by the largest American designers and builders of such houses, a contract has been awarded to the Pierson U-Bar Company of New York for the construction of a building 300 feet long, with a maximum height of 60 feet, divided into three compartments, in which tropical and subtropical vegetation may be planted with effective grouping. The plans provide also for two display-wings in which chrysanthemums, azaleas, cinerarias, cal-

ceolarias, gloxinias, tuberous begonias and other bright-flowered plants may be shown effectively from season to season. This plant house, much needed and destined greatly to enhance the attractiveness of the Garden at all times, but especially during the winter, is expected to be completed by early autumn. Although several years will be required to fill it with imposing plants, it will insure the Garden against the loss of another season's chrysanthemums, and will be a center of interest almost from the day of its completion.

For some years past, the limestone base of the office building,—Mr. Shaw's city home, rebuilt at the Garden about twenty years ago under a provision of his will,—had been deteriorating rapidly. It has now been replaced in granite, with marked improvement in the appearance of the building.

THE HERBARIUM.

Incorporations in the herbarium number 16,594 sheets of specimens, of which 3,073, valued (unmounted) at $153.65, were presented; 773, valued at $38.65, were collected by employees; and 12,748 were bought, the Secretary's books showing an expenditure of $1,211.34 for specimens and material.

The herbarium now consists of:

The Engelmann Herbarium (all groups) . .		97,859
The General Herbarium:—		
Higher plants.		
The J. J. Bernhardi Herbarium .	63,705	
The Henry Eggert Herbarium .	26,704	
The J. H. Redfield Herbarium .	16,447	
The Sturtevant and Smith Herbarium	7,446	
The Gustav Jermy Herbarium .	5,118	
The A. W. Chapman Herbarium .	3,536	
The Julien Reverchon Herbarium	17,210	
The Nicholas Riehl Herbarium .	3,359	
The A. F. Eby Herbarium . .	5,069	
Other specimens	393,452	542,046

Thallophytes.			
The J. J. Bernhardi Herbarium .	4,429		
The Gustav Jermy Herbarium .	1,659		
The S. M. Tracy Herbarium . .	4,393		
The Wm. Trelease Herbarium .	11,000		
Other specimens	37,320	58,801	
Making a total of		698,706	
Valued at			$104,805 90[2]

Other material, supplementing the herbarium, comprises:

Wood specimens, etc., to the value of	$280 00
Microscope slides, etc.	410 00
	$690 00[3]

THE LIBRARY.

Additions to the library consist of 1,353 books, 1,884 pamphlets, 2 manuscripts, and 21,867 index cards. Of these, 917 books, valued at $1,877.75, 1,717 pamphlets, valued at $297.90, and 2 manuscripts, valued at $2.00, were presented; and 436 books and 167 pamphlets were bought, the Secretary's report showing an expenditure of $2,432.31 for purchases and binding. Of the index cards, 13,034 were written by employees, and 8,833 were bought.

The serial publications now received number 1,495, of which 1,389, issued by 1,022 institutions or publishers, are received in exchange for Garden publications and the remaining 106 are bought. The number now reported is 28 more than that noted a year ago.

The library now contains:

Pamphlets	40,375			
Books	28,428			
	68,803,	valued at		$105,825 72
Manuscripts	112,	"	"	1,592 00
Total	68,915	"	"	$107,417 72
Index cards	792,967	"	"	7,929 67
Total valuation				$115,347 39

[2] This valuation at the rate of $15.00 per hundred mounted sheets.
[3] Rept. Mo. Bot. Gard. **16**: 21. **22**: 20.

GARDENING INSTRUCTION.

The course in gardening remains essentially unchanged[4]. The following changes are to be reported in the holders of scholarships: Mr. Edwin Nyden and Mr. Arthur Smith, who had completed the prescribed course, were awarded certificates in March, and entered on work in the line of their profession. On nomination of the St. Louis Florist Club, one of the liberated scholarships was given to Mr. Fred Grossart, of Belleville, Ill., and the other was awarded, on examination, to Mr. Cecil Wakeley, of Louisiana, Mo. In the course of the year Mr. Clark Craig, who had held a scholarship for two years, resigned it to assume the management of a large farm owned by his father. This scholarship was held by Mr. John H. Chilton during the summer, but in the autumn both he and Mr. Wakeley relinquished their scholarships, which are now vacant. The customary notice has been issued in the expectation of re-awarding both in the early spring.

UNDERGRADUATE INSTRUCTION.

No considerable changes have been made in the equipment of the School of Botany laboratories or in the courses of instruction, though both have been somewhat bettered.[4] At the beginning of the present academic year, Mr. G. L. Peltier, a graduate of the University of Wisconsin, was awarded the Teaching Fellowship in botany, held last year by Mr. P. L. Gainey.

Undergraduate enrollment for the first term of 1911-12 is: Botany 1, thirty; Botany 5, one; Botany 7, one; Botany 9, nine; Botany 10, eight; Botany 13, seven; Botany 17, five; Botany 19, two; Botany 21, three; Botany 23, two; Botany 25, five; Special Histology, two; Saturday Course, six:—a total of fifty-one students, each taking one full course in botany.

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[4] Rept. Mo. Bot. Gard. **20**: 32. **22**: 21.

GRADUATE INSTRUCTION AND RESEARCH.

Under the provisions for advanced instruction and research noted in the last two reports[5], five holders of research fellowships have been occupied at the Garden with problems of scientific or economic interest, under the guidance of Professor Moore.

At the last Commencement of Washington University the Master's degree, in botany, was conferred on Miss Frances S. Pickrell, Miss Mildred W. Spargo, Mr. P. L. Gainey, Mr. S. M. McMurran, and Mr. W. W. Ohlweiler; and the Doctor's degree, in botany, on Miss Caroline Rumbold.

There are now registered in candidacy for the Doctor's degree, with botany as a major study, Miss Mildred Spargo and Messrs. C. O. Chambers, W. H. Emig, L. O. Kunkel, W. W. Ohlweiler and Jacob Schramm, and for the Master's degree in botany, Messrs. G. L. Peltier and M. D. Renkenberger. One other candidate for the Doctor's degree takes botany as a minor study, and three graduates, not candidates for a degree, are doing advanced work in botany.

The Rufus J. Lackland Research Fellowships have been awarded for the year 1911-12 to Miss Mildred Spargo and Messrs. C. O. Chambers, W. H. Emig, L. O. Kunkel and Jacob Schramm, of whom Mr. Chambers and Mr. Schramm held fellowships last year. Because of the sustained demand for trained botanists—particularly in the fields of applied physiology and mycology—those who have secured such training promptly find remunerative and responsible employment. Of the Fellows of last year, Dr. Rumbold, Mr. McMurran, Mr. Anderson, and Mr. Gainey passed to responsible positions on leaving St. Louis, as did Miss Hayden and Mr. Arzberger the year before; and with the single exception of Miss Hayden, who is a college teacher of botany, they are giving nearly or quite all of their time to botanical

[5] Rept. Mo. Bot. Gard. **21** : 22. **22** : 22.

research, the results of which can but reflect increasing credit on the Garden, which has been privileged to aid in their development.

During the past year such of the time of capable employees as could be spared for that purpose has been given to research work, and a number of papers embodying the results of such work have been published.

As in former years, the facilities of the Garden have been extended freely to visiting botanists and to correspondents; 14,139 herbarium specimens have been loaned to 28 persons, and 80 books and pamphlets to 21 persons not living in St. Louis, in addition to 134 books loaned to 44 residents of the city.

THE GARDEN STAFF.

No important changes have been made in the library, herbarium or garden staff, except that in the spring Mr. Adolph Jaenicke, a man of large experience in various fields and places, was appointed Foreman to succeed Mr. Otto Bogula who had efficiently held that post for about a year until compelled to relinquish it for personal business reasons.

SPECIAL TESTAMENTARY PROVISIONS.

Three of the annual events provided for in Mr. Shaw's will have taken place in 1911.

The flower sermon was preached in Christ Church Cathedral, St. Louis, on the morning of May 14, 1911, by Rt. Rev. F. F. Johnson, Bishop of South Dakota.

The sum set apart for floral premiums was awarded by the St. Louis Horticultural Society, at its November Exhibition; no award was made of the Shaw medal.[6]

The twenty-second Gardeners' Banquet was given at the Southern Hotel, St. Louis, on the evening of June 15, 1911. Over three hundred persons were seated, two-thirds of them members of the American Association of Nurserymen which

[6] Rept. Mo. Bot. Gard. **5**:18. **9**:19. **11**:20. **16**:29. **19**:23.

was then meeting in St. Louis. The Director of the Garden presided.

The dinner was followed by speeches appropriate to the occasion by Mr. J. H. Dayton, of Ohio, President-elect of the American Association of Nurserymen; Mr. Adolph Jaenicke, of the Missouri Botanical Garden; Mr. J. J. Beneke, Secretary of the St. Louis Florist Club; Hon. Dwight F. Davis, Park Commissioner of the City of St. Louis; Mr. J. W. Hill, of Iowa; Hon. John H. Gundlach, President of the City Council of St. Louis, and Professor J. C. Whitten of the University of Missouri.

Very respectfully,

WILLIAM TRELEASE,
Director.

SCIENTIFIC PAPERS.

A STUDY OF THE PROBLEM OF WATER ABSORPTION.

BY LOUIS OTTO KUNKEL.

I. INTRODUCTION.

When we observe under the microscope a piece of plant tissue, an algal filament, or the spores of a fungus, in that condition that we are pleased to call normal, we find that in each case the cells are quite rigid. This rigidity, or turgidity, as it is generally termed, is due to the fact that the protoplast exerts a certain amount of pressure on its cell wall. When a cell becomes plasmolysed the protoplasm ceases to exert any pressure on the cell wall, and we say that such a cell has lost its turgor.

It is a difficult problem to explain the origin of the force from which results turgidity in living cells. The explanation most generally given is based on the assumption that a semi-permeable membrane or, at least, a membrane possessing only limited permeability for dissolved substances surrounds each cell. When such a membrane is interposed between two solutions of different concentrations, the major current of the solvent is toward the more concentrated solution, and osmotic pressure becomes manifest. In this way is explained the entrance of water into cells. If we assume that a semi-permeable membrane surrounds the living cell, we may account for the pressure observed in turgid cells by saying that it is due to osmotic pressure resulting from a difference in concentration between the solutions inside and outside of the cell. The purpose of this paper is to give the results of some experiments that bear upon this problem and to consider to what extent we are justified in assuming the existence of a semi-permeable living membrane.

This assumption that the cell is an osmotic system carries with it certain conclusions, for osmotic pressure is known to

obey certain laws. When the temperature is kept constant the osmotic pressure of a given solution depends only on the number of dissolved particles in that solution. The chemical nature of these particles has no effect on osmotic pressure; they may be the ions or the molecules of any chemical substance; in other words, osmotic pressure obeys the gas laws. That this simple explanation of turgor and of its cause, the entrance into and retention of water by living cells, is inadequate, becomes evident when one makes a study of the literature dealing with our problem.

II. LITERATURE.

It was long ago observed by Loeb (9) that the amount of water absorbed by muscle tissue when placed in equimolecular solutions of various electrolytes depends on the nature of the dissolved substance. In a certain solution of potassium chloride, for example, the muscle tissue was found to gain from forty to fifty per cent of its original weight after eighteen hours, while in an equimolecular solution of sodium chloride it gained only seven per cent of its original weight. We see that the osmotic theory of the passage of water into cells is not in accord with these observations.

In order to explain such facts and at the same time keep the osmotic theory of water absorption, it has been necessary to suppose that the permeability of the living membrane varies with external conditions. Fernbach (3) showed that dilute alcohol makes the plasma membrane of the yeast cell permeable to invertase. Harvey (6) found that neutral red and other basic dyes fail to enter cells in the presence of a very weak acid. He also found that weak alkalies enter cells, while strong alkalies do not, thus showing that permeability varies with alkalinity. Lavison (7) found that the protoplasm of peas and lupines is permeable to the salts of heavy metals when they are offered in high concentrations, while these same salts do not enter the cells from solutions of low concentration. According to Paine (14) yeast cells take up sodium chloride, ammonium chloride, copper sulphate, and sodium phosphate from moderately concentrated solutions,

but can take up only sodium chloride and ammonium sulphate from dilute solutions. Fluri (5) claims that aluminum salts render the protoplasm of *Spirogyra* cells permeable to ordinary plasmolytic agents. Wächter (18) found that sugar diffuses out of sections of onion in distilled water or in hypotonic sugar solutions, but that the addition of a trace of a potassium salt to the solution prohibits the diffusion of the sugar from the onion tissue. Osterhout (12) has concluded that the antagonistic action of various salts as regards their toxic effects is largely or entirely due to the fact that they hinder the entrance of each other into the protoplasm, *i. e.,* that each salt affects the permeability of the protoplasm for other salts. True and Bartlett (17) have shown that the rate of absorption and secretion by the roots of peas varies with the concentration of the solution in which the roots are immersed and with the nature of the salt or salts dissolved in the solution. McClendon (10) thinks that those substances that stimulate growth do so by affecting the permeability of the protoplasm of the cells stimulated. Czapek (2) found that tannin diffuses from cells of *Echeveria* whenever the surface tension of the medium in which the cells are immersed is lowered to approximately two-thirds that of water. It is claimed by Ramann and Bauer (16) that the time of maximum absorption of an element by the roots of trees varies with the season. The pine, for example, absorbs nitrogen most rapidly in June, while it takes up calcium most rapidly in August.

These observations, made by a number of different workers, show that the membrane, which has been assumed to surround living cells, must, if it exists, alter its permeability under many different conditions. The permeability of non-living membranes is not capable of such alterations. The importance of the supposed membrane surrounding living cells depends entirely, so far as absorption and secretion are concerned, on its degree of permeability. Since the living membrane differs from the non-living membranes in this important characteristic, it naturally follows that we should be very careful when we compare the one with the other. The

effect of a given solution on the turgor of living cells cannot be predicted from a knowledge of the osmotic pressure of that solution. The nature of the dissolved substance is quite important when we are dealing with living cells.

Many facts at variance with the osmotic theory of water absorption have been explained by saying that the living membrane is not quite semi-permeable and that its permeability is different at different times and under different circumstances, but there are certain phenomena that are not to be explained in this simple manner. When the small intestine of a cat was partially filled with a certain watery solution, Cohnheim found that there was a passage of water out of the gut. He explained irreciprocal permeability by saying that two forces are here concerned; one the force of osmotic pressure and the other a force independent of osmotic pressure and located in the living cells.

The secretion from the kidneys contains some substances in higher and some in lower concentration than the blood; it contains less chlorides and more sulphates and urea than does the blood (4). We may explain the passage of urea from the blood to the urine, until its concentration in the latter is equal to that in the former, as due to diffusion, but it is not possible to explain how it becomes more concentrated in the urine than in the blood in this way. Blood corpuscles that take their nourishment from the serum in which they are immersed are rich in potassium phosphate, while the serum is poor in this substance, but rich in sodium chloride (15). Selective absorption has been explained by stating that the substances absorbed are those to which the membrane is permeable, while those that are not absorbed are unable to pass through the living septum. This explanation is very satisfactory until we come to those cases where the final concentration of the diffusing substance becomes greater inside than outside of the cell. This has been explained by supposing that some of the substance, after passing into the cell, enters into combination with some other substance and is thus rendered incapable of diffusion. But there is little evidence to show that this actually takes place. Besides, it has been

found that in certain cases the diffusing substance is less concentrated inside than outside of the cell, although it readily passes into the protoplasm. Paine (14) found that on immersing yeast cells in alcoholic solutions varying in concentration from five to twenty per cent, the ratio of the concentration of the alcohol within the cell to that of the solution outside was practically a constant, and independent of the absolute concentration. The alcohol outside of the cell was always more concentrated than the alcohol inside of the cell. Paine explained this observation by supposing that some of the water within the cell is combined in such a way that it is unable to act as a solvent for alcohol.

The osmotic theory fails us most completely, however, when we come to cases of permanent plasmolysis of cells by substances that readily enter the protoplasm. Lavison (7) found that sodium chloride passes into the protoplasm even from plasmolysing concentrations and causes permanent plasmolysis, although large quantities of the salt are taken up by the protoplasm. He further found that when he plasmolysed cells with an iron salt, the salt, nevertheless, entered the protoplasm, and that if the experiment was not continued too long the cells were not killed by such treatment. How is it possible for a salt that readily passes through the membrane to cause plasmolysis? So far as such salts are concerned the membrane does not exist, and yet we observe a phenomenon (plasmolysis) that is ordinarily explained as due to the action of a semi-permeable membrane.

It must be obvious from the foregoing enumeration of facts recorded and conclusions reached by various workers that diffusion and osmosis do not explain absorption and secretion. There is great confusion regarding the nature of the membrane that has been assumed to surround cells. Lepeschkin (8) has considered it a protein membrane, Overton (13) a lipoid membrane, Nathansohn (11) a membrane in which lipoid particles are mixed with protein particles, while Czapek (2) looks on it as an emulsion of fat, each globule of which is surrounded by a thin layer of soap. All of these things cause us to doubt the value of the assumption that a semi-

permeable membrane or a membrane that is only partially permeable to dissolved substances surrounds living cells.

The literature shows that there are numerous facts that do not accord with the assumption that such a membrane regulates absorption and secretion. But it is certainly not possible to prove that a membrane possessing some degree of semi-permeability does not surround certain cells. The best we can do is to determine the value of the assumption that such a membrane does exist. It was with these facts in mind that a study of this problem was undertaken.

III. EXPERIMENTAL STUDY.

1. Mechanical Injury to the Membrane.

Since the permeability of living protoplasm is quite susceptible to change, and since its permeability is supposed to result from the character of a living membrane surrounding the protoplast, one must naturally expect this membrane to be easily capable of mechanical injury. The following observations bear on this phase of our problem:

When one places vigorous filaments of *Spirogyra setiformis* (Roth) Kütz., which have been growing in pond water into a strong solution (solutions about two times molar were used) of potassium or sodium chloride and observes them with a low-power microscope, the cells do not appear to be plasmolysed, but when they are observed more closely and stronger magnification is used one sees that they are very much plasmolysed. The protoplast now occupies only a small fraction of its original volume and forms one or more spherical masses within the cavity surrounded by the chlorophyll bands. If the cells are carefully observed during the course of this experiment it will be seen that when the strong chloride solution surrounds them they become plasmolysed very quickly. The chlorophyll bands often occupy exactly the same position after plasmolysis that they occupied when the cell was in its normal condition. If a weaker solution is used, plasmolysis takes place more slowly and the chlorophyll bands are displaced toward the center of

the cell by the contracting protoplasm. When this happens the bands generally lose their spiral form and appear as irregular masses of green.

Thus, when the cells are plasmolysed by a strong solution, the chlorophyll bands are not displaced and lie, after plasmolysis, entirely outside of the protoplasm. If, on the other hand, a weaker solution is used and plasmolysis is brought about more slowly, the chlorophyll bands are carried toward the center of the cell and lie, after plasmolysis, entirely within the protoplasmic mass. When the cells plasmolysed by a strong solution are observed under a low-power microscope, they do not appear to be plasmolysed. The chlorophyll bands still occupy their normal position, and the spherical protoplasmic masses being quite hyaline are difficult to distinguish.

When the cells are plasmolysed by a strong solution, the membrane surrounding the protoplasm must be broken in many places in order to allow the escape of the chlorophyll bands. If the membrane is differentiated from the remainder of the protoplasm, *i. e.,* if there is really a membrane surrounding the protoplast, one would expect it to lose its semipermeability after such treatment. Certainly no inert membrane with which we are acquainted could be used to demonstrate osmosis after being broken in so many places. The spherical masses of protoplasm that have been freed of their spiral bands will, nevertheless, take up water and enlarge if the solution in which they are immersed be diluted with distilled water. No success has accompanied efforts to make the spherical masses enlarge sufficiently to fill the entire cell. They always burst and disintegrate before they have enlarged to that extent. These protoplasmic masses take up water, although any membrane which may have surrounded the cell in its normal condition must have been broken when the chlorophyll bands passed out through the surface layers of the protoplasm. This experiment was often repeated, and always with the same results.

If a membrane surrounds these *Spirogyra* cells in their

normal condition, then, either the integrity of that membrane is not lost when it is broken or a new membrane is formed about the protoplasm very soon after the old one is destroyed. The spherical masses within the plasmolysed cells show themselves capable of taking up water, even when the solution is diluted immediately after plasmolysis. It is hard to think that the short period of time necessary to dilute the plasmolysing solution is sufficient to allow of much differentiation in the outer layers of the protoplasm. If we dispense with the idea that a new membrane is formed immediately after the old one is disrupted, then the only conclusion to which we can come is that the membrane surrounding the normal protoplast, if such a membrane exists, is not very susceptible to mechanical injury. One would expect to find a living membrane more delicate than non-living membranes, yet we do not know of an inert membrane through which solid bodies may be forced without rendering it quite permeable.

2. The Concentration of Cell-Sap.

Along with the assumption that a semi-permeable membrane surrounds the living plant cell, goes the further assumption that the solution contained within the membrane is as concentrated if not more concentrated than the liquids surrounding the turgid cell. If this were not true the cell would not remain turgid, but would lose some of its water and become plasmolysed. If, therefore, cell-sap be obtained from cells that are as nearly alike as possible it should represent the concentration of the solution originally within the cells. If cells like those used in this experiment be placed in the sap, they should remain practically normal as regards their turgor. But if this sap solution be concentrated it should then plasmolyse these cells. In order to determine whether or not this would take place, the following experiment was performed:

Monilia sitophila (Mont.) Sacc. was grown on pieces of potato that had been washed in running tap water for forty-eight hours. From these cultures fifty-two and one-half grams of spores were collected. They were obtained by inverting the cultures and shaking them above a piece of paper.

They were harvested three weeks after the cultures were started. Spores were taken only from pure cultures of the fungus. They were ground up in a mortar with fine quartz sand. Before being used this sand was boiled in alkali, then in acid, and finally in several changes of distilled water, in order to free it of impurities. The entire mass of spores after being ground up in this way, was subjected to a high pressure, and several cubic centimeters of sap were squeezed out. This sap solution was reddish-yellow in color, had a specific gravity of about 1.053, contained absolutely no reducing sugars, was neutral to litmus and gave, on the addition of alcohol or when heated above 50° C., a heavy floculent precipitate.

Some spores and mycelial cells exactly like those used for obtaining sap were placed in this solution. After sixteen hours none of the cells showed plasmolysis; most of them had germinated and the solution was filled with mycelium. Cells of *Spirogyra setiformis,* on the other hand, became quickly plasmolysed when placed in the sap of the *Monilia* spores. Some of the sap solution was boiled down to one-tenth of its original volume. In this concentrated solution the spores and mycelial cells became quickly and permanently plasmolysed. The exact concentration of the sap that would be just sufficient to plasmolyse the cells was not determined, but it was found that even after considerable concentration the sap solution would not cause plasmolysis. This result indicates that the *Monilia* cells do not behave as ordinary osmotic systems; if they did we should expect them to become plasmolysed in solutions of their own sap, even when such solutions are only slightly more concentrated than the sap. In slightly concentrated solutions of their cell-sap the spores germinate and grow. From this it seems that spores of *Monilia* are able to take up water from solutions more concentrated than their own cell-sap.

As was mentioned above, the sap solution was of a reddish yellow color. It is interesting to note in this connection that when the spores are placed in the sap solution they readily take up the coloring matter and become a much deeper color

than the sap itself. Even in the sap solution concentrated to one-tenth of its original volume, the spores, though they became plasmolysed, absorbed so much of the coloring matter that they appeared quite yellow when observed under the microscope. They became a much deeper color than the solution that surrounded them. This is an interesting case of selective absorption, and one in which the substance absorbed is a product of the cells that show the absorption. Moreover, the coloring matter becomes more concentrated inside than outside of the cell. This, like other examples of the same phenomenon, cannot be explained as due to diffusion. Diffusion would tend to equalize the concentration of the coloring matter inside and outside of the cell. If a mass of *Monilia* cells are placed in a small amount of water and left for several hours, the water does not become colored. None of the coloring matter diffuses out of the cells. When, however, the cells are placed in a solution of *Monilia* cell-sap they quickly take up the coloring matter and become a much deeper color than before. Thus it seems that the reddish yellow substance contained in *Monilia* cells that are grown in the light is capable of passing into, but not out of, these cells. A semi-permeable membrane does not help us to explain this observation.

3. Absorption of Water by Egg-Albumen.

If a small amount of egg-albumen be placed in a paper bag and suspended in distilled water, the albumen will take up water and the bag will gain in weight. Much less weight is gained when such a bag is suspended in a strong solution of sodium or potassium chloride. A number of experiments have been performed for the purpose of determining the effect of different concentrations of sodium chloride on the absorption of water by egg-albumen. In all of these experiments the egg-albumen was taken from fresh hen's eggs, placed in small paper bags and immersed in the solution to be tested.

After bag No. 1 had remained in a 2M sodium chloride solution for eighteen hours and had gained only 1.2 per cent of its original weight, as shown by the table below, it was re-

moved from the sodium chloride solution and suspended for 20 hours in distilled water. At the end of this period of time it weighed 41.5 grams, having gained more than 10 grams during the 20 hours in distilled water. Bag No. 4, which, as shown by the following table, had gained 44 per cent of its original weight during the 18 hours that it was suspended in distilled water, was, at the end of this period of time, removed from the distilled water and placed for 20 hours in 2M sodium chloride solution. At the end of 20 hours it weighed 37.4 grams, having lost 1.7 grams during the 20 hours that it was suspended in the 2M sodium chloride solution. At the end of the experiment No. 1 had gained 43 per cent of its original weight, while No. 4 had gained only 36 per cent of its original weight. During the 20 hours in distilled water, No. 1 gained 41.8 per cent in weight, while during the same period of time No. 4, in 2M sodium chloride solution, lost 5.3 per cent in weight.

The following table shows the results of such an experiment:

Number on bag	Weight of bag	Weight of bag plus albumen	Concentration of solution used	Weight of bag after 18 hours in solution	Per cent gain in Weight
1	6.9 gr.	31.0 gr.	2 M NaCl	31.3	1.2
2	7.0 "	26.9 "	M NaCl	29.7	14.0
3	7.1 "	30.6 "	$\frac{M}{2}$ NaCl	34.8	18.0
4	7.1 "	29.3 "	Distilled water	39.1	44.1

The egg-albumen used in this experiment is certainly quite different from living protoplasm, and yet it is the substance that we ordinarily think of as being most like protoplasm. The white of the egg is a colloidal solution of albumens. As Lavison (7) has pointed out, protoplasm is principally a colloidal solution of albumens. The paper used to separate the egg-albumen from its surrounding medium in the above experiment differs in some respects from the cellulose wall that surrounds ordinary plant cells. Nevertheless, the two are much alike. The paper differs from the cell wall in physical structure, but the two are quite similar chemically. The paper, like the cell wall, is permeable to both water and electrolytes.

While this artificial cell differs from the living plant cell, the two are alike in several ways. The artificial cell takes up water from a dilute solution, but gives up water when transferred from a dilute to a concentrated solution. The living cell takes up water from dilute solutions, but gives up water when placed in concentrated solutions. The layer of egg-albumen in contact with the paper bag certainly does not become differentiated so as to form a semi-permeable membrane, for egg-white is miscible with water in all proportions.

Osmotic pressure is sometimes supposed to be demonstrated by breaking some of the shell from one end of an ordinary hen's egg and then placing it in a tumbler of water. After a period of time the membrane surrounding the egg bulges at the point where the shell was removed, thus showing that a pressure has developed inside of the membrane. The membrane surrounding the egg may not be permeable to certain dissolved substances, but a paper bag that is quite permeable to these will serve just as well as the membrane when we wish to show that egg-albumen will take up water from dilute solutions. The pressure developed within the egg when it is placed in a tumbler of water is, for the most part, I believe, not a result of osmotic pressure, but a pressure due to the affinity that the colloids in the egg have for the water outside of the egg. Sometimes, in experiments like this, so much water is taken up that the membrane becomes greatly distended and finally breaks. At this we need not be surprised. Fisher has shown that certain colloids have a strong affinity for water.[1]

IV. DISCUSSION.

That diffusion enters into the problems of absorption and secretion by living cells cannot be doubted, but that such cells maintain their turgor by virtue of osmotic pressure and are surrounded by semi-permeable membranes is pure assumption. We can explain turgor and water absorption without assuming the existence of a living membrane possessing the

[1] Fisher has shown that the colloids in the eye of the sheep are capable of taking up water from certain solutions until so much pressure is developed that they burst the strong coat surrounding the eye.

property of semi-permeability (4). Moreover, such an explanation seems to accord better with facts than does the explanation based on the assumption that such a membrane exists. On the whole, the pressure developed in living cells does not obey the gas laws. In order to keep the osmotic theory of water absorption it is necessary to make various assumptions regarding the changes in the permeability of the assumed membrane. When we assume that a semi-permeable membrane exists, and undertake to become familiar with its properties, we find that they are not known. The permeability of the same cell seems to be affected differently by different substances. It changes as the concentration of the medium changes and seems to vary, even with the season of the year. The semi-permeable living membrane is an assumption, upon which is based a theory, which, it seems to me, is not only no longer useful, but even detrimental to a correct understanding of the phenomena of absorption and secretion. As Martin H. Fisher (4) has pointed out, we know very little regarding the affinity between colloids and watery solutions. We use the word "affinity" to cover our ignorance, but it seems better to do this than to make an assumption that does not find justification in the facts that are known.

I am greatly indebted to Dr. George T. Moore for the encouragement and advice that he has given me while engaged in this work.

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A CONSIDERATION OF THE PHYSIOLOGY AND LIFE HISTORY OF A PARASITIC BOTRYTIS ON PEPPER AND LETTUCE.[1]

BY GEORGE L. PELTIER.

INTRODUCTION.

During the autumn of 1911, while gathering the peppers *(Capsicum)* in the vegetable plat of the Missouri Botanical Garden, the writer observed a fungus which was appearing as a parasite on the fruits. The peduncles were covered with a dark brown mass of conidia and mycelium, which so weakened the tissues that the fruit soon fell to the ground. On opening the peppers a number of large, flat, crust-like sclerotia, 1 cm. long and .5 cm. wide, were found to fill the interior. In most cases the seeds were also covered with the crust-like masses. The conidial stage was identified as *Botrytis cinerea* Pers. (Botrytis vulgaris Fr.). Although *Botrytis cinerea* has been observed parasitic on a large number of hosts, this is the first time it has been reported as a parasite on the peppers.

Later, while carrying on infection experiments, it was introduced into one of the vegetable greenhouses, where it became quite a serious disease of the lettuce, causing the characteristic "drop" and a "damping-off" of the seedlings. Thus a very good opportunity was afforded for the study of the development of the parasite and its action on several hosts.

HISTORICAL.

By using a part of the artificial classification suggested by Duggar (17), the genus *Sclerotinia* may be divided into four distinct groups:—

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[1] Abstracted from a thesis presented to the Faculty of Washington University, in candidacy for the degree of Master of Arts, June, 1912.

1. Species comprising in their life cycle not only apothecia, but also a monilia stage, *i. e.,* with conidia produced in chains, the latter frequently separated one from another by special structural devices known as disjunctors.[2]

This group may be further divided into two subheads:— *(a)* Species in which both spore forms may be produced upon the same host; *(b)* species whose life cycle is not complete on a single host.

2. Species which may embrace a form of *Botrytis* as a conidial stage. Under this head we may include forms which produce sclerotia and which germinate to produce conidia directly, the apothecial stage having been entirely lost.

3. Species in which no conidial stage has been convincingly demonstrated.

4. Species which produce neither conidia nor apothecia.

Under *(a)* of the first group may be included forms like *S. vaccinii* (Wor.), Rehm. on *Vaccinium vitis idaea, S. oxycocci,* Wor. on *V. oxycoccus* and *S. fructigena,* Schröeter on *Prunus,* whose life history and development have been so well worked out by Woronin (69). Under *(b)* may be included *S. heteroica,* Wor. and Nawasch. on *V. uliginosum* and *Ledum palustre* and *S. rhododendri,* Fischer on *Rhododendron ferruginosum* and *Rhododendron hirsutum.* It is interesting to note that these fungi are truly parasitic, but that in all forms attacking species of *Vaccinium,* infection takes place through the flower where the spores, while germinating can live saprophytically on the stigmatic exudation.

The second group includes only one species, *S. Fuckeliana* DeBary, which in its conidial stage is the well-known facultative parasite, *Botrytis cinerea* Pers. De Bary (15) first

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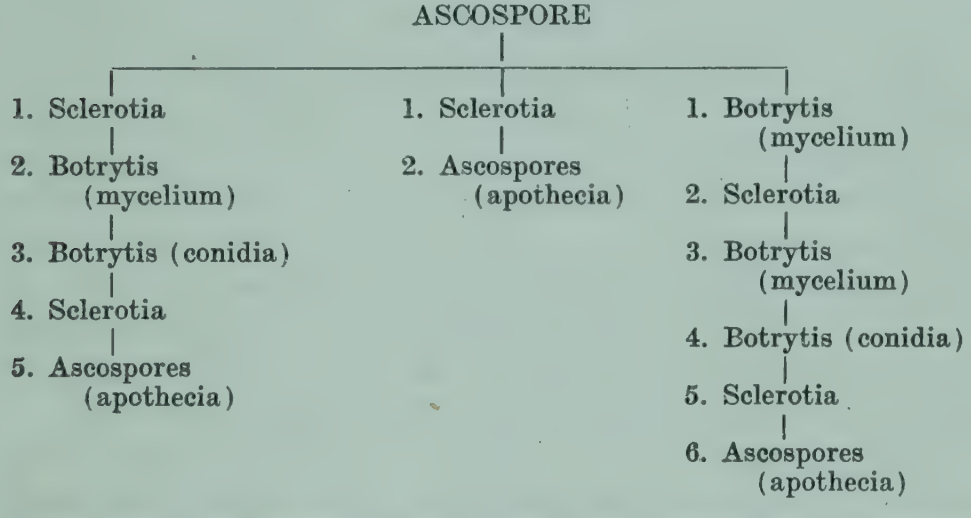
[2] Thom, in a recent paper, throws a new light on the origin of these devices. He states that the newly-formed conidium is cylindrical, and quickly swells to assume a spherical or elliptical form. During the process the connective appears when the primary wall of the original tube fails to follow the change in form and leaves an open space between itself and the new walls of the adjacent conidia in the chain. (The connective between conidia of *Penicillium.* Science, **35** : 149. 1912.)

connected the conidial stage of *Botrytis* with the apothecial form produced from the sclerotia of *Botrytis* on grape. However, up to the present time this connection has been disputed, as many observers have failed repeatedly to secure, under favorable conditions, the perfect stage from sclerotia produced by *Botrytis*. The utmost confusion exists since many authors have tried to connect the botrytis stage with *Sclerotinia Libertiana* Fuckel, whose conidial stage has probably been completely lost.

In Europe, *Botrytis cinerea* is important as the cause of a disease on a number of hosts, but it is especially destructive, at times, on the leaves and fruit of the grape. Pirotta (39), in 1881, again connected *Sclerotinia Fuckeliana* with the imperfect stage of *B. cinerea,* and was also able to prove that *S. Libertiana* was distinct from the above forms. The material was found on the fallen leaves and fruit of the grape. The work of Pirotta can be summed up by adding the following table, which he gives in his summary:—



This table of Pirotta, starting with the ascospore, represents the three lines of development which he was able to trace.

Brefeld (9) at first accepted the results of De Bary, but later he discredited them, being unable to obtain apothecia from the sclerotia after many attempts, germination resulting always in the production of conidia. Viala (61) and Ravaz (43), in France, and Brizi (10), in Germany, described a rot

of the grape shoots and grafts due to *Botrytis*. Numerous sclerotia were produced on the stems. Later, in 1897, Prillieux (41) was able to produce ascospores from the sclerotia formed on the shoots of grape. Tubeuf (61), after a number of attempts, obtained only conidia, while Zopf (71) found that he was able to produce apothecia after the sclerotia remained dormant at least one year.

During the next few years a number of writers in France, Germany and Italy described maladies affecting different parts of the grape plant. Finally, in 1905, Istvanffi (28), in an excellent paper, established the connection of *Botrytis cinerea* with *Sclerotinia Fuckeliana*. The author finds that the botrytis form of the fungus lives saprophytically on fallen leaves, twigs and fruits, during the early season. The conidia develop with great rapidity on the arrival of warm, moist weather and, when transferred to the growing foliage, germinate to produce a parasitic mycelium. The *Botrytis* attacks the ripening fruit in autumn, often causing great loss. Sclerotia are formed abundantly on the fruits and also on the cuttings made during the summer. The fungus may be carried over the winter in stratified cuttings and young shoots.

Istvanffi finds that the sclerotia develop on every part of the plant, appearing as small yellow papilla, consisting of a gelatinous mass which soon becomes hard and darker in color. The mycelium passes through the wood by way of the medullary rays, and sclerotia may be formed under the bark of the stems and shoots.

It is sufficient to say that the sclerotia are of the cartilaginous type, composed of two parts, a cortical layer, formed by the swelling of the filaments at the surface, and the pseudo-parenchyma inside, formed by the coalescing of the mycelium. During the formation of the sclerotia large drops of a colorless fluid are exuded. In addition to the larger sclerotia, small cone-shaped, striated pseudo-sclerotia are formed which, after a brief period of rest, germinate in the fall to produce large tufts of conidiophores.

The sclerotia germinate in the fall or late spring in one of two ways; either producing conidiophores from the swollen branches of the hyphae or from the stromatic tissue, which is formed as a sort of pseudo-parenchyma on the surface of the sclerotia.

The production of apothecia from sclerotia Istvanffi found to take place anywhere on the surface, from two to six developing from a single one. The apothecia arise by the breaking down of the cortical layer and the pushing out of the swollen hyphae in parallel tubes. At this point the hyphae grow out and branch, forming the cup of the apothecium. The process takes place, in some cultures, in less than nine days after the sclerotia are placed in moist sand. Repeated infection experiments by the above author with ascospores resulted in failure, although they germinated in water. However, by using bits of the apothecia he was successful even on the leaves and fruit of the grape.

The origin and functions of the organs of attachment are discussed at length. The author finds that they serve several purposes, such as attaching the fungus to the host, breaking the cuticle of the fruit and stem and forming, together with the mycelium, tufts which are able to live over the winter.

A disease of hemp, similar to that of lettuce, has been reported from Europe on several occasions. The infected stems are permeated with mycelium, which soon causes the entire collapse of the plant. Behrens (5) found both *Botrytis cinerea* and *S. Libertiana* present on the above host, and at first believed them to be stages of the same fungus. However, he subsequently concluded that the two forms were probably distinct. Tichomiroff (58) observed a disease of hemp having the same symptoms which he attributed to a new *Sclerotinia,* called by him *S. Kaufmaniana.* De Bary was able to infect the hemp plants with the ascospores of *S. Libertiana,* and decided that the disease described by Tichomiroff was nothing more than *S. Libertiana.* Later Hazlinsky (25), in Hungary, and Hiltner (26), in Germany, noticed a disease of the hemp identical with the above. In

both cases the disease was attributed to *Botrytis,* no sclerotia appearing on the plants. It would seem that we have a case where both forms, *Botrytis* and *S. Libertiana,* are associated, but entirely distinct.

In a like manner Frank (18) noticed a disease of rape, and found both *Botrytis cinerea* and *Sclerotinia Libertiana* present, consequently concluding that the two stages were of the same fungus. De Bary, however, discredited Frank's conclusion as to the connection of the two stages. It appears that, like the disease of hemp, we have the two fungi associated, though distinct.

Again, in a rotting of the stems of the potato in various parts of Europe, the same confusion exists. After reviewing the literature on this subject the identical conclusions were reached; that is, the two forms, *Botrytis* and *Sclerotinia Libertiana,* although causing the same symptons and generally associated, are always distinct forms.

In Holland the confusion of the different forms is still greater, as a separate species is made for every new host. However, here again the two forms stand out distinct. They are associated on the same host, causing a widespread disease of the tulip and other bulbous plants. The *Botrytis,* which is not typical *B. cinerea* on account of the short branching conidiophores which it produces, is known as *B. parasitica* Cav., while the sclerotia, ascribed to *S. bulborum* Wak., resemble *S. Libertiana.*

Other epidemics, in Europe, of some magnitude have been ascribed to attacks by *Botrytis* on a number of fruits, vegetables and greenhouse plants resulting, in most cases, in a complete rotting and collapse of the host.

A number of diseases caused by *Botrytis* alone have been reported from Europe at various times, the lily disease in England, observed by Ward (66), is one of the best known examples. The *Botrytis* attacking the lily, while not typical *B. cinerea,* is at least of the *B. cinerea* type. Kissling (31), in the same year, reported at length on *Botrytis* as a parasite on *Gentiana lutea,* the horse chestnut and a number of greenhouse plants.

Under the name *B. Douglasii,* Tubeuf (59) describes a disease of the Douglas fir *(Pseudotsuga Douglasii).* It is characterized by the withering, curling up and death of the shoots toward the tips of young seedlings and the lower twigs of older trees. In the fall, sclerotia about the size of pinheads break through the epidermis under the old bud scales and on the needles. When placed in a moist chamber tufts of conidiophores immediately arise from the sclerotia. The conidia germinate directly in water and infect young plants, which are killed in a few days. Tubeuf also reports this same fungus on *Prunus triloba* and *Juniperus communis.*[3] Ritzema Bos (45) found that *B. Douglasii* was parasitic on a number of conifers. Smith (48), while working in Europe, observed a similar infection on the linden, attributing it to *B. cinerea.*

Brooks and Bartlett (12), in England, described a disease of the gooseberry due to *Botrytis,* its chief characteristic being the ability to penetrate the woody tissues of the plant. The first indication of the trouble is the wilting of the foliage, followed by the death of the branches attacked. Sclerotia were found beneath the bark, and in some cases conidia.

Botrytis cinerea seems to be the less frequent cause of the lettuce "drop" in the greenhouse, although it is a more serious parasite than is generally supposed. Since Humphrey (27) reported it in Massachusetts in 1892, it has been observed in at least ten states, causing more or less loss. The disease is especially destructive in the lettuce-growing centers along the Atlantic coast. Up to 1900, *Botrytis cinerea* was supposed to be the sole cause of the "drop." However, Smith (48), who had been working on the problem for a number of years, found that most of the"drop" in Massachusetts was not due to *Botrytis,* as generally believed, but to *S. Libertiana.* Up to the present time there is still considerable doubt as to which form is the primary cause, although it seems that both fungi are capable of infecting the lettuce under favorable conditions.

Humphrey (27) described a typical attack of *Botrytis* on

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[3] From his figures, *B. Douglasii* appears to be identical with *B. cinerea.*

greenhouse lettuce, and from his description there is no evidence whatever that *S. Libertiana* was present. In the same year, Jones (29) in Vermont mentions a lettuce rot due to a *Botrytis*. Under the term "mildew," Taft (57), in 1894, gives an account of a lettuce disease, attributing it to a species of the same genus. Galloway (19) states that "wet rot," produced by *Botrytis,* is the result of too much moisture. Bailey (2) believed that the lettuce "rot" was caused by *Botrytis,* which is at first saprophytic on decaying matter in the soil, but under conditions unfavorable to the plant, attacks it and acts as a parasite. He figures a plant infected with the fungus which is characteristic of the "drop." Selby (47) mentions a disease as lettuce "rot," attributing it to *Botrytis.*

Stone and Smith (54), in 1897, describe a "drop" due to *Botrytis.* Their description of the disease agrees very well with that of Humphrey. Again, in 1898 and 1899, they refer to the lettuce "drop", still attributing it to the same fungus, which produced a number of small flat sclerotia on the infected plants and in the cultures. Garman (20) at this time refers to a "rot" of lettuce due to *Botrytis* and other organisms. It is interesting to note that the disease causing the so-called "drop" was in all cases attributed to *Botrytis cinerea.*

Smith (48), in 1900, in a paper in which he brings together a large number of observations, concludes that there are two distinct forms which cause the lettuce "drop." The first he designates as the Botrytis type which, besides causing the "drop," produces a damping-off of the young seedlings. The fungus growing in the cultures and on the plant produced an abundance of conidia with but little mycelium. Small sclerotia were formed in large numbers, both in the cultures and on the infected plant. The second type he refers to as the "no-Botrytis" type, belonging to the fungus *S. Libertiana.* This form produces a cottony mass of mycelium with sclerotia, but no conidia. Infection takes place directly by the mycelium. Smith, unable after numerous attempts to germinate the sclerotia, was of the opinion that the bulk of the disease is due to a degenerate form of this

no-Botrytis type, while *Botrytis* causes the "drop" only in rare instances, on account of its inability to attack vigorous plants under normal conditions.

Ramsey (42), several years later, described a lettuce "drop" caused by *Botrytis* during the fall in one greenhouse and by *S. Libertiana* in late winter, in a second. The former was characterized by a sort of watery decay, followed by the production of conidia, while the latter produced an abundance of sclerotia and a loose cottony mycelium.

In the past year Stevens and Hall (53) attribute the lettuce "drop" in North Carolina to *S. Libertiana.* They were able to germinate the sclerotia readily and found that infection was brought about by the ascospores as well as by the mycelium. Both ascospores and mycelium are comparatively short-lived, even under favorable conditions, so that they do not, to any great extent, serve as a means of carrying this fungus over considerable periods of time. The sclerotia, on the other hand, are long-lived and are capable of carrying the fungus over unfavorable seasons, so that the ascospores are able to produce infection on the next crop of lettuce. Here, also, the ascospores are for a short time, at least, saprophytically nourished before they infect the plants. Contrary to Smith's observations, the mycelium did not appear abundantly on the soil and was unable to migrate any distance through it. No mention whatever is made of the fungus *Botrytis cinerea.*

Botrytis cinerea has been reported several times in this country on greenhouse plants. Atkinson describes and figures a *Botrytis* parasitic on carnations. Halsted (22) remarks that *Botrytis,* previously nourished on the blossoms, is the cause of the decayed patches upon many greenhouse plants. Spaulding (52) described carefully the disease on chrysanthemums, *Euphorbia pulcherrima* and *Primula obconica grandiflora.*

The two best known fungi which may be included under the third group are *S. Libertiana* Fuckel and *S. trifoliorum* Eriks. The latter has always been regarded as an entirely

distinct form with no conidial stage. It is occasionally very destructive to species of clover *(Trifolium)*. It is regarded by some to be identical with *S. Libertiana.*

The utmost confusion exists with regard to *S. Libertiana,* and it is generally associated with *Botrytis.* However, we may accept De Bary's work as conclusive that the former is always distinct and has no conidial stage whatever. In Europe it has been reported on a large number of hosts. In the United States it is certainly one of the causes of the lettuce "drop."

S. rhizoides may be taken as an example of a *Sclerotinia* which produces neither conidia nor apothecia. Stout (56), in a recent publication, states that in no way has he been able to induce germination of the sclerotia.

MORPHOLOGY.

As has already been mentioned, the first indication of the disease on the pepper is the wilting and subsequent browning of the leaves and stems. Later the stems show numerous tufts of conidiophores of *Botrytis cinerea* bursting through the bark. Careful examination shows that the conidiophores arise from the mycelium which permeates the stem. The peduncles of the fruits are also covered with a dark mass of conidia. About this time the tissues of the peduncle are so weakened that the fruits fall to the ground. Mycelium and conidia are present at the point of attachment, but otherwise the fruits seem to be quite healthy. However, on opening the fruits, a number of flat sclerotia are found filling the interior. Little mycelium is present. The sclerotia are very large; some of them, in fact, being over 1 cm. in length and half as wide. These were preserved during the winter and attempts made to germinate them.

When the stems, from which the conidiophores of *Botrytis* arise, are cut transversely, a characteristic discoloration of the wood is evident. The whole stem, with the exception of a narrow peripheral zone of light color, is stained a dark brown. Upon cutting longitudinal sections of the diseased stem, the

well-marked hyphae of *Botrytis* are seen ramifying abundantly. Very few sclerotia are produced in the stems.

Since the infection occurred late in the year, it was impossible to make further observations as to the parasitic nature of the fungus. There can be no question but that the warm, wet weather of October had some influence on the virulence of the disease. The *Botrytis* was obtained in pure culture and it was while trying infection experiments on young seedlings of the pepper that it was introduced into the greenhouse, causing a destructive disease of the lettuce.

The vegetable greenhouse was given over almost entirely to the growing of lettuce during the winter months. The epidemic was first observed in February, the most obvious symptoms being the drooping of the outer leaves, followed in a day or so by the complete collapse of the plants into a slimy mass. The first evidence of the disease was a number of small brownish spots on the stem next to the ground and on the lower leaves. This was soon followed by the wilting and drooping of the outer leaves. A day or so later the whole plant was affected, the outer leaves falling to the ground, only the central head remaining erect. The leaves at this stage had the appearance of being scalded. A few days later the whole plant collapsed into a fetid, slimy mass. The above description is characteristic for most of the infected plants.

At times other plants, to all external appearance healthy, fell to the ground. In this case the fungus worked its way up through the stem into the center of the head, developing there and finally working its way out to the exterior. This method of infection was rather common. Again another type was occasionally noticed, one side of the plant rotting away, while the opposite side remained unaffected.

No conidia are produced until marked decomposition has been brought about within the leaves by the mycelium. The conidiophores are visible to the eye as small brownish masses scattered over the decayed parts. The most profuse development of the conidia takes place on the under side of the leaves

and in other protected places where there is an abundance of moisture.

Little mycelium is to be found on the decayed plants, although mycelium with conidia was abundant on the soil throughout the beds, growing on dead organic material. On examining the tissues of the lettuce, the mycelium appears comparatively large and coarse, varying in diameter somewhat, with many septa and no regular method of branching, except when forming organs of attachment. The latter are formed abundantly in cultures and on the plants, appearing as numerous short branches from near the tip of an ordinary hypha. Each primary offshoot produces branches that again branch, thus forming a compact tuft. At first they are continuous with the main axis, but later become separated by a wall. On touching any object they flatten at the tips, forming disk-like appressoria which adhere to the surface.

The sclerotia do not develop until the lettuce plant has practically decayed. They vary in shape, the smaller ones being spherical, while the larger are, as a rule, irregularly cylindrical or flat. In size there is also much variation, the smaller ones being no larger than the head of a pin, the larger ones the size of a pea. The number of sclerotia produced on one plant varies considerably; fifty-two, unequal in size but all well-developed, were found on one plant. The sclerotia are of the cartilaginous type with an outer black cortical layer and in an inner white, closely packed pseudo-parenchyma, formed by the pressing together of the mycelium.

Sclerotia obtained from plants produced only tufts of conidiophores, no apothecia being obtained. Sclerotia left lying on the soil in the houses gave the same results. The conidiophores are produced by the growing and pushing out of the ends of hyphae in the pseudo-parenchyma, and may be formed on any part of the sclerotium. The conidiophores and conidia which arise in this way are in no respect different from those arising directly from the mycelium.

On moist sand the sclerotium germinates in about two days, the development of the conidia being very rapid. This

method, then, is efficient in the production of rapid infection, while the sclerotia serve to tide the fungus over adverse conditions.

Young lettuce seedlings, planted in flats, were on several occasions destroyed by *Botrytis*. Here the symptoms were practically the same as in the "drop," except that the stems were encircled near the ground by the mycelium and cut off, producing a typical "damping-off." Conidia and a few sclerotia were developed from the mycelium, which lives saprophytically on the dead tissues of the fallen seedlings.

In the same manner, tomato seedlings in a number of flats were destroyed by the fungus, causing the characteristic "damping-off."

In November the poinsettias *(Euphorbia pulcherrima)* were infected by a *Botrytis*. In general, the symptoms of the disease were similar to those described by Spaulding (52), the flowers, green leaves, and in some cases even the colored leaves of the involucre being attacked. The fungus seemed to flourish on the stigmas, the spores, after germination, apparently living for some time saprophytically on the stigmatic exudation before becoming parasitic. The mycelium soon permeated the flower clusters, which fall off after a short time. In falling, the flowers strike the leaves and in some cases adhere to them, bringing about conditions favorable for new infections. On the leaves the disease first appears as light brownish spots. The fungus seems to attack the leaves in such a manner that the latex of the plant is excreted through the diseased spots and hardens on exposure to the air. Here again the mycelium first lives as a saprophyte and later becomes parasitic. The excretion of latex is the most characteristic symptom of the disease, as it is found on all plants attacked.

Another peculiar characteristic of the disease is that the infected areas are localized, considerable portions of the leaf remaining free from the parasite. Finally the petiole becomes weakened and the leaf drops off. In this way the whole stem is defoliated, leaving only the red-colored leaves at the

top, which are less easily affected. In the later stages, conidia arise from the diseased areas. This *Botrytis* did not appear to be typical *B. cinerea,* because of its low manner of growth and the short branching conidiophores. However, when the fungus was obtained in pure culture and grown on suitable media, sclerotia were produced which showed that it was *Botrytis cinerea.* It also resembles in many ways the *Botrytis* described by Ward, which is in all probability a form of *B. cinerea.*

During the month of May the *Botrytis* in the greenhouse, where the infection experiments were tried, was in some manner carried over to a neighboring house given over to the growing of garden beans, where, under conditions favorable to its development, it attacked the flowers and pods. The spore germinating on the flowers developed into a parasite, and the diseased flowers falling on the pods rapidly spread the disease. Several times during the spring *Botrytis* has been observed on plants killed by a light frost; here conidia were formed abundantly, but no sclerotia.

PHYSIOLOGY.

The fungus was obtained in pure culture by plating on potato agar. From a rather abundant growth of mycelium conidia were transferred to suitable media in test tubes. All subsequent cultures from lettuce and other sources were secured in the same manner. The fungus has been kept in pure culture since October, 1911, and its behavior on various media studied to ascertain the one most suitable to employ in experimental work. All cultures, unless otherwise stated, were grown at room temperature.

Media: Upon lettuce leaves in Petri dishes, a rather vigorous mycelium, with many conidia and few sclerotia, was produced.

In lettuce broth, mycelium developed abundantly, but no conidia or sclerotia were produced until the culture was rather old and the mycelium had formed a thick mat on the surface. In no case were conidia developed in the liquid itself.

Tomato, pepper and apple broths gave about the same results, the mycelium growing equally well in all the media. Potato broth furnished a better medium, for, after the mat of mycelium was formed on the surface, conidia and sclerotia were produced in abundance.

On a two per cent lettuce agar, growth was rapid for about two days, afterwards decreasing slowly. In seven days the medium was covered with a thin layer of mycelium, which then produced conidia. Only a few sclerotia, about the size of a pinhead, arose from the hyphae. A similar growth was obtained on apple, pepper and tomato agar. Growth on potato agar was more vigorous, conidia and numerous sclerotia being formed. The spherical sclerotia, as a rule, were larger than the others mentioned above, being the size of a small pea.

Very little mycelium was obtained on nutrient gelatin and bouillon, no sclerotia and only a few conidia developing. When the study of the parasitism of the fungus was undertaken, a large number of cultures were made on sugars, fats and acids. The results of this work will be discussed under parasitism.

Sterilized corn-meal, saturated with various juices, gave good results, inducing rapid development of sclerotia. Apple, lettuce, tomato, pepper, potato and prune juices were used. Corn-meal moistened with potato and prune juices furnished the best medium for growth. Later, sterilized bread-crumbs were substituted for the corn-meal, as it was found to produce mycelium much more abundantly. In all of the above cultures growth was not rapid at first, but in five days the mycelium covered the entire surface as a loose, white, cottony mass composed of large, branching, septate hyphae. Organs of attachment were produced in large numbers wherever the mycelium came in contact with the sides of the dish.

The crowding of the mycelium led to the formation of a rather regular zone of sclerotia. As the cultures became older the latter united with one another, so that the zonate appearance was lost to some extent. The sclerotia matured in from

nine to twelve days and in fifteen days formed a mass, or crust.

Five flasks, containing bread-crumbs saturated with potato broth, were inoculated February 1, 1912. On February 19, the first sclerotia were gathered. Then every three days the sclerotia were collected for a period of two months, when the medium became exhausted. An average of 512 sclerotia was obtained during this time for each culture, a surprisingly large number.

When the first cultures on bread were started, conidia developed at once, either before or while the sclerotia were forming. However, on making transfers repeatedly, the number of conidia became less, while the sclerotia increased in number proportionately. Thus cultures were obtained with very few conidia, which did not develop until all of the sclerotia had matured and the cultures had begun to dry up. This condition was noticed in cultures of the fungus obtained from several sources. When inoculations were made with such conidia on a medium containing little nutriment, conidia were immediately produced on the mycelium, but no sclerotia. It appears, then, that the conidia and sclerotia bear a definite relation to one another; that is, the formation of sclerotia requires a considerable amount of mycelium, whereas the conidia are produced when the mycelium is poorly developed and conditions for growth are unfavorable.

Acidity and alkalinity: The best development of the fungus took place on a slightly acid medium. A strongly alkaline medium retarded growth considerably, causing the mycelium to remain sterile. On the other hand, a strongly acid medium reduced the number of sclerotia, but favored the production of conidia.

Temperature: Cultures of the fungus on bread-crumbs saturated with potato broth were subjected to the following temperatures: 37°C., 30°C., 20°C. and 15°C.

From the results obtained it was clearly evident that a temperature of 37°C. was unfavorable for the growth of the fungus. Growth stopped entirely after progressing a few millimeters, no conidia or sclerotia being produced. At

30°C. the mycelium grew moderately well, covering the surface in about three weeks. At the end of five weeks the experiment was discontinued, the mycelium remaining sterile. At ordinary room temperature the best growth took place; numerous sclerotia were formed, with the conidia developing, as a rule, after the sclerotia had, for the most part, matured. Growth was somewhat retarded, but after two weeks the mycelium completely covered the surface of the plate and sclerotia developed. Only in one of the cultures were conidia produced, the abundant mycelium giving rise only to sclerotia.

From these experiments we may conclude that the fungus is unable to live long at a temperature of 37°C.; that at a temperature of 15°C. and 30°C. growth of the mycelium takes place normally, but not rapidly; a few sclerotia and, in some cultures, conidia are produced. The optimum, then, for rapid normal growth of the mycelium lies in the neighborhood of 20°C.

What the significance of the non-production of conidia at certain temperatures is we are unable to explain. Beauverie (3), working on the polymorphism of *Botrytis,* obtained a similar result. He succeeded in getting a strain of the fungus which, when kept under certain conditions (mainly of temperature and moisture), never formed conidia. He found that after controlling these factors for a long time the fungus was unable to return to its former state. When the above conditions were varied it always remained sterile.

Light and darkness: No appreciable difference was noted between the cultures grown in light and in darkness, except that the latter favored the non-production of conidia and the formation of a large number of the striated pseudo-sclerotia, while light favored the greater development of conidia. The sclerotia appeared to grow equally well in light and in darkness.

Longevity: The length of life of the mycelium varies but little, and in no case does it extend beyond two months. The conidia, on the other hand, are much longer-lived, since even after six months they are capable of germinating and pro-

ducing infection. The sclerotia are not as long-lived as generally supposed, for, under suitable conditions, they germinate soon after maturity, which power decreases directly with the age of the sclerotia. When kept in moist soil for three months a large number disintegrate, leaving the hard, black exterior. But few sclerotia live more than a year. The pseudo-sclerotia produce conidia after a short period of rest; those that do not germinate rot in a few weeks, none surviving to the next season.

Germination of the sclerotia: From sclerotia developed on the lettuce and pepper plants a large number of cultures were started early in December, so that by January almost two thousand sclerotia were available. Those obtained from the peppers in the fall were kept in a dry place for three months. At this time two hundred cultures which had been gradually accommodated to the lower temperature were placed out of doors. The "drop" in the greenhouse being at its height, a large number of sclerotia were obtained from this source also. Some of them were left on the soil for further observations.

The first series of experiments was started January 15. Sclerotia from the above sources were placed in flasks containing sterilized sand moistened with sterile water. A similar experiment was set up every two weeks until May 15, a period of four months. Beginning March 1, there were added to the series sclerotia placed outside during the winter months.

In no case were apothecia formed, germination always resulting in the production of tufts of conidiophores. The results of these experiments may be summed up by saying that in from two to six days, depending on the age of the sclerotia, germination took place by the production of conidiophores bearing large masses of conidia, in no way different from those arising from the vegetative hyphæ. Cultures inoculated with these conidia gave a typical development, mainly the production of a loose, cottony mycelium which formed both conidia and sclerotia anew.

The sclerotia, after producing a number of these tufts, dis-

integrated and disappeared, none remaining intact. The large majority of them germinated, the others rotting within three months.

Thinking that possibly the sclerotia could be induced to germinate otherwise than by conidia, various chemical agents were used to stimulate them. Sclerotia obtained from the above sources were used in these experiments. One series was placed in a saturated atmosphere of chloroform for twenty-four and forty-eight hours. This was repeated for ether. Others were placed in desiccators, where they remained from one to seven days. The sclerotia were then placed in flasks as before.

Eighty-five per cent of the sclerotia placed in chloroform and ether vapors and fifty per cent of those exposed for forty-eight hours formed tufts of conidiophores. Germination, as before, was rapid. The cultures were allowed to run for three months, but no further development was observed. Conidia obtained from all the flasks and placed on media showed no differences in growth and development. Sclerotia, when mature, placed on moist sand, again formed the characteristic tufts of conidia. Careful observations in the field and greenhouse showed no further development of the sclerotia other than the production of conidia. About April 15, the sclerotia placed in the field formed conidiophores.

From the numerous experiments outlined above it follows that the fungus, *Sclerotinia Fuckeliana,* with *Botrytis cinerea* on lettuce as its imperfect stage, has lost its apothecial stage entirely, the life cycle being complete when the conidia, produced by the sclerotia, germinate to form a vegetative mycelium.

Germination of the conidia: The conidia germinate readily in water and nutrient solutions. Germination by one, sometimes two, germ tubes is the rule. In two days the mycelium is quite profuse and if the germinating spores are in hanging drops, organs of attachment are formed almost immediately. The conidia from the sclerotia germinate in like manner. Three generations of conidia have been observed, no difference

whatever in their germination and development being noted. Sclerotia when sectioned and placed on culture media are able to produce mycelium which produces normal conidia.

Infection experiments: In no case was infection observed on healthy plants unless drops of water were present on the leaves; therefore, the plants were moistened with water just previous to inoculation. Plants sprayed with conidia in a nutrient solution like potato and prune broth produced infection in the majority of cases. Inoculated plants covered with bell jars gave almost a hundred per cent infection, the disease appearing in about three days. The progress of the disease was more rapid on cloudy days and when ventilation of the greenhouse was poor. Inoculations into wounds with spores produced the disease in all cases.

Old lettuce plants, when dusted with dry conidia, were very slightly affected; when water was allowed to run into the lettuce head the percentage of infection was much greater. Lettuce plants sprayed with conidia in water or in a nutrient solution produced, as a rule, a heart rot. During the winter months lettuce was planted from time to time in the infected beds which, in a short time, became diseased. In addition to the distribution of the disease by conidia, the mycelium, which was found to some extent in the soil, was also capable of producing infection.

With pepper and tomato seedlings no infection took place unless the spores were applied suspended in a nutrient solution and the plants covered with bell jars. In most cases the inoculated plants showed the presence of the fungus in three days, and within a week the plants collapsed. During the month of May, when the house was open and the days clear, all the above experiments were repeated, but negative results were obtained in most cases, showing that under conditions unfavorable to the fungus very little infection occurs.

Experiments with the fruit of the tomato showed that in no case could the germ tube, produced by the conidia, penetrate the cuticle, even when they were submerged in a nutrient solution. When the cuticle was broken, the conidia

germinated and entered the tissues of the tomato. Here it either remained in the tissues, where it produced a rot, or the mycelium appeared on the surface and developed numerous conidiophores. The rotting of the fruit in the latter case was much slower. Carrots, potatoes and turnips, inoculated with conidia, rot slowly. The surface of these vegetables, if broken during the progress of the disease, became covered with a dark mass of conidia in less than twelve hours.

The fungus, then, was able to produce direct infection in the majority of cases if water was present on the plant. It was able to propagate itself, and cause the "drop," by its conidia, as well as by the mycelium. Infection by the mycelium always occurred on the leaves nearest to or on the ground. Infection experiments in the field were a failure in the majority of cases.

Parasitism and Saprophytism: Because the fungus exhibited so many degrees of parasitism in the infection experiments, it seemed desirable to make a rather detailed study of this subject. This same question has troubled a number of authors and conflicting results have been obtained.

De Bary (14), working with *Sclerotinia Libertiana,* found that the ascospores were not capable of producing direct infection, but must be saprophytically nourished before they possess the power of penetrating the living plant tissues. He believed that some substance, or substances, was secreted by the hyphæ, which dissolved the tissues of the host. Two phenomena could be distinguished in the breaking down of the tissues; the death of the cell contents and the destruction of the cell walls accomplished by a cellulose enzyme which dissolves, swells and breaks down the middle lamella of the cell walls.

Later, Kissling (31), investigating the development of *Botrytis cinerea,* like De Bary, found that the conidia were unable to bring about direct infection of the leaves of *Gentiana lutea,* although the young mycelium attacked the flowers. This peculiarity has been pointed out before, as one of the characteristics of the *Sclerotinia,* causing mummified fruits,

and agrees with the observations of the disease on poinsettias. As was stated, this infection is due to the saprophytic nourishment afforded to the germinating spores by the stigmatic secretions. Kissling assumes that a cellulose dissolving enzyme is the sole cause of the destruction of the cell walls and the transformation of the cell contents into available food.

On the other hand, Ward (66) found that direct infection was possible in the form of *Botrytis* investigated by him. However, he observed that the infection was more virulent if the fungus was first saprophytically nourished. Like the others, he attributes the disorganization of the tissue to a cellulose enzyme secreted by the fungus. He further observed that this was accomplished by a marked swelling of the middle lamella, which preceded the breaking down of the cell walls.

Nordhausen (37), investigating the biology of *Botrytis cinerea,* was unable to make out this swelling of the middle lamella. He found that the non-cuticularized, etiolated and injured plants were much more susceptible to infection.

Smith (49) believes that too much importance has been given to this cellulose dissolving enzyme. He finds that we have two stages in the process of the breaking down of the living tissues; first, a poisoning and killing of the cells, due probably to oxalic acid; second, their disintegration and utilization by the fungus. The work of Brooks (11) can be summed up in his statement that, "Whatever may be the causes at work in the living cells which confer immunity or predisposition on the species of the host plants, or which confer virulence or impotence on the spore, they lie deeper than nutrition." We may safely say that *Botrytis* varies greatly in its ability to be a true parasite, and that in most cases it is at first saprophytic.

Tissues infected by *Botrytis* become soft and decay is very rapid. Sections of the diseased tissues show a darkening, loss of turgidity, breaking down of the cell contents, separation of the cells from one another and final disintegration of the whole tissue into a slimy mass. As was observed by Smith

(49), large areas surrounding the mycelium appear to be dead, showing that the fungus secretes some substance which kills the tissues in advance of the hyphæ. Strictly speaking, then, the fungus is always a saprophyte, because it invariably lives on dead tissue, and its ability to penetrate living tissue bougie, was quite concentrated, free from any foreign substance secreted by the hyphæ.

To carry this point a step further the writer obtained an extract from the mycelium and spores by collecting them from the cultures and grinding the material with clean quartz sand in a mortar. To obtain as concentrated an extract as possible, only a few cc. of water were added to the mass and the whole placed in a hydraulic press. The extract, passed through a bougie, was quite concentrated, free from any foreign substances and sterile.

A petiole of the lettuce submerged in the extract darkened and disintegrated very rapidly; acting for a longer time, weaker concentrations gave the same results. The point that attracted attention was that the tissues one to two centimeters above the liquid, were discolored and fell to pieces rapidly. On boiling the extract for varying lengths of time and placing the petioles of lettuce leaves in them the same characteristic changes in the tissues occurred. Sections of the tissues showed that the microscopical changes were in all cases similar to those observed in the diseased lettuce leaves.

Working along the line suggested by Smith, the next step was to ascertain the nature of the substance that caused the disorganization of the plant cells. Using several delicate tests for oxalic acid, negative results were obtained in each case. These were repeated a number of times with the same result.

Solutions of oxalic acid of different concentrations when applied to the petioles showed an action on the tissues somewhat like that of the extract, except that the tissues were bleached instead of becoming darker in color. Solutions of acetic, gallic, malic and tartaric acids of the same strength as the oxalic acid used, affected the tissues of the petioles in much the same manner. It may be, then, that the substance

secreted by the fungus is an organic acid whose nature remains to be determined. That it is not oxalic acid, as is generally supposed, seems to be shown by the numerous experiments performed. Smith (49) states that he found as high as two per cent of oxalic acid in old culture media, but does not state how he demonstrated its presence. The great difficulty in this work is to distinguish sharply between the organic acids likely to be found in the plant.

The second part of the work was to determine what enzymes digested the food after the tissue was killed. Attempts were also made to ascertain whether the same enzymes were secreted on different media. Accordingly, a mineral nutrient solution (Pfeffer's) was used as a stock solution.

Two strains of the *Botrytis* were used in the experiments, one that had been obtained in pure culture from the lettuce in the greenhouse, the other secured in March, from the old infected stems of the pepper. Thus one was growing as a saprophyte, the other as a parasite.

A two per cent starch solution with the stock solution was used, which formed a paste. Growth was slow for the first two days but at the end of two weeks surpassed all of the other cultures in the production of sclerotia. In the cultures inoculated with the second strain, more conidia and fewer sclerotia were formed, which was the rule in all of the other cultures; no other differences were noted. At the end of two weeks the medium gave no reaction with iodine. Fehling's solution showed a strong reduction. With Barfoed's reagent a positive reaction was obtained, and with phenylhydranzine a brownish osazone was precipitated. The crystals, when microscopically examined, appeared to be glucosazone, which was further substantiated by their insolubility. The results show that a large amount of diastase is secreted by the fungus. Smith (49) reports that he was unable to obtain any growth whatever on starch, possibly due to some toxic substance in the commercial corn-starch used by him.

As a check on the above work, the action of the extract on a starch solution was observed. Using equal volumes of starch

solution and extract, no iodine reaction could be obtained after an hour, although Fehling's solution gave negative results. However, at the end of twelve hours, hydrolysis had taken place and the final product appeared to be dextrose. The slow growth on the starch medium at first, also shows that hydrolysis must take place before it can be digested by the hyphæ. A three per cent solution of dextrose gave a vigorous growth from the beginning. The mycelial development was luxuriant, many sclerotia and some conidia being formed.

Growth on cane sugar like that of starch was rather slow at first. By the end of the first week the development equaled that in the dextrose culture. Tested in the usual manner, a strong reduction was observed, showing that inversion had taken place. As before, the extract produced similar changes in the medium.

Sugar solutions of various concentrations were inoculated with *Botrytis* conidia. Growth resulted in all cultures, the twenty and thirty per cent solutions giving the best development. Delicate tests failed to show the presence of any oxalic acid whatever.

With maltose an excellent growth developed, producing sclerotia and conidia. Lactose gave a fairly good growth after the first week. On levulose an abundant mycelium, similar to that on dextrose, developed. On dextrin, growth was slow at first, but soon a vigorous mycelium was produced. A strong reduction was noted in the cultural solution and in the medium after treatment with the extract.

In nearly all cases, Fehling's solution shows the presence of reducing sugars in originally non-reducing carbohydrate media upon which *Botrytis* has been grown, showing that a number of enzymes are secreted by the mycelium. No oxalic acid is formed in the cultures, contrary to Smith's (49) results. Careful observations were made from time to time, but in no case was alcoholic fermentation detected.

On glycerin, normal conidiophores were not produced. On testing for oxalic acid negative results were again obtained.

Tests for cellulose digestion were made with filter paper, ground to a pulp, and absorbent cotton, to both of which a stock solution was added. On the pulp a fair development of the mycelium resulted, with the production of spherical sclerotia, while on cotton only a slight growth was observed. These experiments were repeated several times, as so many authors have attributed the breaking down of the cell to a cellulose enzyme. Fehling's solution showed only a slight reduction. The mycelial extract was allowed to act on pulp for a week, and when tested gave only a slight reduction. It may be assumed, therefore, that very little cytase is formed and that too much importance has been attributed to the rôle of this enzyme.

A two per cent solution of castor oil produced a development not altogether normal. The oil lost its characteristic appearance and broke down into glycerin and fatty acids.

Malic and tartaric acids gave a fair growth of mycelium, with few sclerotia and conidia. With oxalic acid no development whatever took place, even in low concentrations.

With a two per cent solution of tannin, growth resulted in only one of six cultures and the effect of *Botrytis* on this substance was not followed out, except that the culture was finally observed to turn black. Smith found that the tannin was broken down into glucose, gallic acid and a dark coloring matter which he was unable to identify.

Amygdalin gave an excellent development of mycelium and conidia, but no sclerotia. Again no attempt was made to study the effects of the fungus on the medium.

A solution of peptone produced an excellent growth and no changes in the medium could be detected.

All of these experiments show that most of the carbohydrates, fats and protein derivatives used can serve as the sole carbon source for the fungus and that probably substances in the plant cells belonging to the above classes may be digested by the enzymes secreted by the hyphal elements. The poisoning of the plant cells may be due to one or a num-

ber of organic acids, or it may be that the fungus secretes some other toxic substance which causes the death of the cells.

DISCUSSION.

There can be no question but that the disease on the peppers, lettuce and other plants in the greenhouses of the Missouri Botanical Garden was caused by *Botrytis cinerea,* which possessed the power of penetrating the plant tissues and destroying them.

The lettuce "drop" due to *Botrytis cinerea* has about the same characteristic symptoms as when caused by *Sclerotinia Libertiana.* It is, indeed, interesting to note the similarity between the symptoms of the disease described by Stevens and those noted by the writer. Even the physiology of the two fungi agree to a large extent. The difference, of course, is that *Botrytis* produces only conidia, whereas *S. Libertiana* only ascospores. In this connection I might say that I have been able to develop from *Botrytis* a mycelium that does not produce conidia. From my observations it appears that the more parasitic the *Botrytis* becomes, the greater is the number of sclerotia and the fewer the conidia produced.

That *Botrytis* can become a serious disease was very well shown in the greenhouse the past winter. To become parasitic, however, it must have suitable conditions. The life cycle of the fungus is complete with the germination of the sclerotia, resulting in the production of conidiophores. This appears to be the prevailing type of sclerotium germination. Istvanffi (28) is the only one who in recent years has been able to obtain ascospores from sclerotia of *Botrytis.* Since he was unable to produce infection by means of the ascospores, he failed to complete the life cycle of the fungus. Apothecia were rare; the sclerotia formed conidiophores more commonly. It may be safely stated, then, that the *Botrytis* causing lettuce "drop" and similar diseases is a degenerate form which has lost the apothecial stage.

Work on the parasitism of *Botrytis* is still in its infancy and many further experiments will be necessary before the interrelations with its host are fully understood. My results show that we have two stages in the disintegration of the plant tissues. There can be no question that some substance is secreted by the fungus which kills the plant cells in advance of the mycelium. The author disagrees with Smith (49) that the poisoning effect is due to oxalic acid, since delicate tests for this acid gave only negative results. Even in old sugar cultures on which the fungus has been growing for several weeks, no oxalic acid was present. Smith reports that he found as high as two per cent of the acid under like conditions.

The harmful substance may be some organic acid other than oxalic, or it may be a toxin of some kind, which, however, is not destroyed by heating to 100°C. The writer has found that weak concentrations of malic, tartaric, oxalic, gallic, and acetic acids have an action on the lettuce tissues similar to that of the mycelial extract.

From the cultural work I have found that a number of enzymes are secreted by the fungus. These enzymes are diastase, invertase, cytase and lipase. It was also shown that glucosides were broken down and that the fungus was able to live on protein derivatives. The *Botrytis* investigated secretes very little cytase and it seems that too much importance has been attributed to this enzyme. It may be that diastase assists the cytase in breaking down the cellulose and in digesting the amylodextrin formed. In agreement with the other authors, the experiments show a varying ability of the conidia to cause direct infection of the host. This ability is always directly proportional to the conditions favoring the fungus.

SUMMARY.

1. The *Botrytis cinerea* causing the diseases of lettuce, peppers and greenhouse plants mentioned, at the Missouri Botanical Garden, is the imperfect stage of *Sclerotinia*

Fuckeliana De Bary. It is a degenerate form, having lost the apothecial stage entirely, the life cycle being completed when the sclerotia germinate by the production of tufts of conidiophores.

2. In all cases *Sclerotinia Fuckeliana* has no connection whatsoever with *Sclerotinia Libertiana* Fuckel.

3. Two stages in the parasitism of the fungus have been noted: first, the killing of the cells in advance of the fungus, due to some organic acid (not oxalic) or to some toxin (not destroyed by boiling) secreted by the fungus; second, the digestion of the dead tissues by a number of enzymes which are produced in varying quantities by the fungus.

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EXPLANATION OF PLATES.

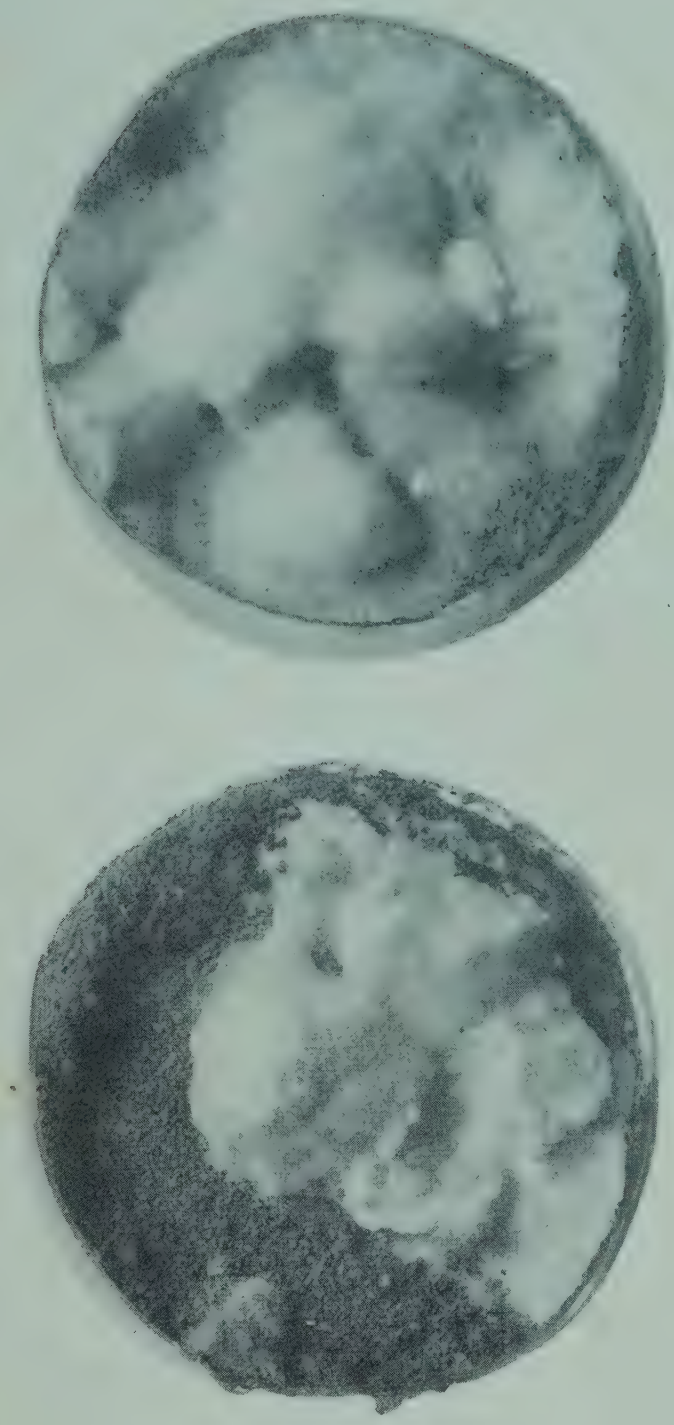
1. Two typical cultures of *Botrytis cinerea* on bread-crumbs saturated with potato broth, showing abundant growth of mycelium and developing sclerotia. No conidia are present in the cultures, although a few were formed later. Cultures are eight days old.

2. A pure culture of the fungus on bread-potato broth mixture kept in the refrigerator at a temperature of 15°C. for five weeks. A number of sclerotia are scattered through the culture but conidia are lacking.

3. Tomatoes inoculated with conidia, showing conidiophores arising at the point of inoculation.

4. *Botrytis cinerea* infecting beans.

5. Germinating sclerotia, showing the formation of large tufts of conidiophores.



BOTRYTIS CINEREA.

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BIOMETRIC DATA ON THE INFLORESCENCE AND FRUIT OF CRINUM LONGIFOLIUM.

BY J. ARTHUR HARRIS.

I. INTRODUCTORY REMARKS.

Crinum longifolium is familiar to botanists through wide cultivation or through numerous descriptions and figures. At fruiting time, the plants present a remarkable appearance, the massive fruit clusters bending the stalks to the ground, which is strewn with the peculiar seeds. These are exceedingly large and fleshy—fine examples of the bulbiform seeds of the Amaryllidaceae.

Consider some of the problems in the physiology of seed production presented by the inflorescence, fruits and seeds. The form and size of the fruits is closely related to the peculiarities of the seeds. It is neither an elastically dehiscent capsule nor a berry, but resembles a leathern bag, which finally softens to spill out its contents. The number and size of the contained seeds, and consequently the size of the fruit, varies enormously. Some of the seed bags are as large as apples, while others are small sterile knobs terminating the end of the pedicel. In number the seeds range from none to seventy, or more, per fruit; in weight, from nothing to nine grams.

If there are differences in the amount of available plastic material from inflorescence to inflorescence, or from fruit to fruit, or if there is (as has been suggested in biological literature) a competition between the various primordia for the available material, these factors should, it seems to me, be especially easily recognized in structures showing such wide extremes.

These problems attracted my attention several years ago. Partly owing to difficulties inherent in the material, partly

because of the pressure of other work at the fruiting season, I have never been able to complete fully the study as planned in 1905 and 1906. At present, I see no opportunity of returning to it. It seems worth while, therefore, to put on record the data collected and analyzed, together with suggestions toward further work along these lines.

Summarily stated, our problems are:—

(a) To describe certain series of material in terms of biometric constants and their probable errors. These, besides being necessary to the solution of the following problems, may serve as a basis of comparison for fertility characters in other species.

(b) To determine, by comparison with constants for other forms, whether the morphological characters of the reproductive organs in *C. longifolium* can be considered to have any influence upon their variability.

(c) To determine whether the capacity of the inflorescence for maturing its ovaries into fruits is in any degree dependent upon the absolute number of flowers which it produces.

(d) To determine whether the capacity of the ovary for maturing its ovules into seeds is in any degree dependent upon the number of fruits per inflorescence.

(e) To obtain some measure of the differences in the capacity of the inflorescences for maturing ovaries into fruits, ovules into seeds, or for forming large seeds.

II. MATERIAL.

All of the material came from a fine row of *C. longifolium* in the Missouri Botanical Garden[1], which is yearly loaded with the characteristic fruits. In the spring of 1905, I *(a)*

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[1] Dr. Trelease has kindly called my attention to the following point: "The type form is somewhat tinged with red and most of our collection is of this form, but here and there occur white-flowered plants of the var. *album*. I do not believe that you differentiated these when your collections were made and yet I do not believe that they will in any way affect your general results."

counted the number of seeds in a random sample of 1,000 fruits, *(b)* weighed a series of 333 seeds individually, *(c)* determined the number of flowers formed and number of fruits developing as well as the number of seeds per fruit in a series of individual inflorescences.

Collections were again made in 1906. In 309 inflorescences, the number of seeds per fruit was determined in all fruits possible.[2] A lot of 2,000 seeds was weighed.

III. CONSIDERATION OF PROBLEMS.

1. Variation in Number of Flowers and Number of Fruits per Inflorescence.

Data for number of flowers formed and number of fruits developing per inflorescence are given in the totals of the correlation tables for 1905, Table VII, and for 1906, Table VIII. The constants are given in Table I.

TABLE I.

	1905	1906	Difference
Flowers			
Mean.....	10.668 ± .126	11.518 ± .101	.850 ± .161
S. D.....	2.594 ± .089	2.626 ± .071	.032 ± .113
C. V.....	24.315 ± .883	22.795 ± .650	1.520 ± 1.096
Fruits			
Mean.....	5.689 ± .093	7.065 ± .086	1.376 ± .127
S. D.....	1.917 ± .066	2.242 ± .061	.325 ± .090
C. V.....	33.699 ± 1.282	31.731 ± 1.291	1.968 ± 1.819

[2] It is almost impossible to collect inflorescences at a time to secure the well-developed pods without including some which have lost part of their seeds. There is considerable variation in the time of flowering and a few inflorescences are still in flower when the bulk of the materials must be collected. The later-flowering inflorescences probably produce fewer or smaller fruits, but I have no means of making a quantitative estimate of the difference. The material collected both years was taken almost, if not quite, entirely from the maximum period. Our material is, therefore, to some extent selected. Thus while the statistical investigation of this form is of very great interest, it is surrounded by many difficulties; the results must be interpreted with caution. Some of the difficulties met are discussed in their proper context.

The differences between the two series are of precisely the kind which one would expect from the errors of random sampling, and from slight seasonal influences. The constants are chiefly of importance as a basis for the calculation of correlations. It is interesting, however, to note for comparison with other characters, to be discussed later, that the variability in neither of these cases is at all large. Compare the coefficients of variation with those available for other inflorescences, in Table II. It will be shown that variation in both the number of seeds per fruit and the weight of the seeds is very much higher in *C. longifolium* than in most other species. This is probably referable to the large number of primordia formed and the large size attained by the seeds. Taken together, these render the development of a high proportion of the ovules impossible. Some of the fruits produce a very large number of seeds while others tend to be sterile. Some of the seeds attain a very large size, but the majority must remain small. The immediate interest of these points lies in the fact that no influence of the peculiarities of seed formation upon the type or variability of the parts of the inflorescence can be detected.

TABLE II.

RELATIVE VARIABILITY IN INFLORESCENCES.

Species	Authority and Place of Publication	Coefficient of Variation	
		Flowers	Fruits
Cichorium Intybus.....	De Helguero, Biometrika. **5** : 185.	10.28 — 10.98	.....
Staphylea trifolia.....	Harris, Biometrika. **6** : 441.	64.44	53.04
Cornus Mas.....	Ritter, Beih. Bot. Centralbl. **25** : 21.	17.83 — 29.72	.....
Adoxa Moschatellina...	Harris, Biometrika. **7** : 220.	6.95 — 14.42	.....
Nothoscordum striatum	Harris, Rep. Mo. Bot. Gard. **20** : 108.	22.44 — 25.51	.....
Allium stellatum.....	" " " " " **20** : 112.	31.79	.....
Celastrus scandens	" " " " " **20** : 118.	26.80	48.58
Cercis canadensis.....	Harris, unpublished data.	12.09 — 24.97	.....
Sanguisorba officinalis..	Ritter, Beih. Bot. Centralbl. **25** : 7-8.	38.54 — 39.96	.....
Anethum graveolens ...	De Vries, The Mut. Theory. **1** : 558-561.	24.07 — 26.50	.....
Coriandrum Sativum...	" " " " **1** : 571.	14.37 — 14.48	.....

2. The Distribution of Fertility in the Fruits.

The fertility of a fruit is measured by the number of seeds which it produces. Table III gives the number per fruit and the frequencies for:—

(a) A random sample of 1,000 fruits taken in the spring of 1905.

(b) The 768 fruits of the 143 inflorescences with no dehisced fruits collected in the spring of 1905.

(c) The 1,245 fruits comprising the five fertile fruits each from 249 inflorescences of 1906.

(d) The 1,331 fruits from the random selection of eight rays from each of 280 inflorescences in 1906.

TABLE III.

Seeds	a	b	c	d	Seeds	a	b	c	d
1	108	151	57	64	36	10	2	10	16
2	124	104	83	93	37	3	3	11	11
3	108	49	94	100	38	4	2	9	12
4	68	48	74	69	39	2	1	8	5
5	46	40	67	63	40	2	1	6	6
6	34	43	42	43	41	1	2	7	4
7	50	29	45	54	42	2	1	9	5
8	43	29	57	59	43	1	4	9	7
9	36	21	35	46	44	2	.....	6	7
10	26	14	49	48	45	5	2	7	4
11	25	15	31	39	46	.....	.....	2	3
12	37	20	31	35	47	1	1	2	3
13	22	10	26	24	48	1	.....	2	2
14	19	15	28	40	49	.....	.....	3	3
15	20	16	27	25	50	2	.....	4	5
16	26	18	24	23	51	.....	2	2	1
17	12	12	25	30	52	.....	.....	5	3
18	6	8	28	27	53	1	1	1	4
19	12	7	30	26	54	.....	2	2	2
20	16	9	20	27	55	.....	.....	1	1
21	7	13	24	22	56	.....	.....	3	1
22	12	8	22	26	57	1	.....	1	1
23	19	6	23	19	58	.....	.....	1	1
24	8	14	20	27	59	.....	.....	2	.....
25	11	7	12	17	60	.....	.....	.....	1
26	5	9	20	20	61	.....	.....	1	1
27	12	7	20	20	62	.....	.....	.....	.....
28	11	3	17	22	63	.....	.....	1	.....
29	9	4	17	17	64	.....	.....	1	1
30	3	3	12	16	65	.....	.....	1	1
31	4	2	20	15	66	.....	.....	1	1
32	10	3	12	19	67	.....	.....	.....	.....
33	5	1	13	17	68	.....	.....	1	1
34	7	2	9	13	69	.....	.....	.....	.....
35	1	4	11	12	70	.....	.....	1	1

The series *(c)* and *(d)* bear to each other the relation of random sub-samples. Some of the fruits in the two series are identical. The two are inserted merely to show the reader how considerable irregularities may arise through errors of random sampling. Series *(a)* and *(b)* are both samples from the same population, but taken in a somewhat different manner. Diagram 1, in which the heights of the several vertical lines represent the frequencies for the 57 classes of fruits, gives the 1905 random sample. The statistical constants are given in Table IV.

TABLE IV.

Series	Mean Seeds	Standard Deviation	Coefficient of Variation
(a) 1905.....	10.338±.221	10.342±.156	99.55
(b) 1905.....	9.242±.246	10.095±.174	109.23
(c) 1906.....	15.471±.225	13.344±.180	86.25
(d) 1906.....	15.283±.239	12.941±.169	84.67

Two characteristics of these distributions are remarkable. The range of variation is exceedingly wide. The mode lies far to the lower end of the range, the most frequent cases being fruits with but a single seed or, at most, three seeds.

DIAGRAM I.

The frequencies of various numbers of seeds per fruit are represented by the heights of the ordinates.

A comparison of the frequencies in Table III reveals dissimilarities too great to be attributed solely to the probable errors of random sampling.[3] Even the two samples of 1905 seem to differ far more, in the frequencies of the first three classes at least, than can be explained by the errors of sampling from a homogeneous population. Series *(a)* seems to have a significantly higher mean than series *(b)*. The difference is over thrice its probable error and confirms the impressions from the frequency distributions. Probably the difference in the method of collection of these two lots of material introduced some unknown biological factor to which the differentiation is due.[4] In the 1906 series, the empirical mode is on 3 instead of 1 or 2. The mean and standard deviations are also higher and the range is wider than in the 1905 collections.

The coefficients of variation show how exceedingly great the variability in this material really is. The reader will appreciate this by a comparison of the constants for several other species of plants, as given in Table V.

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[3] How great such differences may be for individual classes may be seen by a comparison of the frequencies in series *(c)* and *(d)*. Naturally, the reader will remember that these are actual, not relative, frequencies, and that in one case, N = 1245, while in the other, N = 1331.

[4] The difficulties of collecting really typical samples of material (already discussed in an earlier section), while at the same time retaining data for all the characteristics which it was desired to consider, was one of the chief reasons for discontinuing the study of *Crinum*.

TABLE V.

VARIABILITY IN NUMBER OF OVULES AND NUMBER OF SEEDS PER LOCULE (l) AND PER FRUIT (f).

Species	Authority and Place of Publication	Ovules per Fruit	Seeds per Fruit
Sanguinaria canadensis	Harris, Biometrika. **7** : 309-310.	30.28 — 30.66	32.66 — 39.26
Robina Pseud-acacia...	" " **6** : 442.	18.69	45.45
Hibiscus militaris (l)...	" " **8** : 55-64.	.....	20.70
" lasiocarpos (l)	" " **8** : 55-64.	.....	32.88
" Moscheutos (l)	" " **8** : 55-64.	.....	18.93
" Trionum (l)..	" " **8** : 55-64.	17.49	22.58
" syriacus (f) ..	" " **8** : 55-64.	8.79 — 10.21	27.65 — 46.78
" " (l) ..	" " **8** : 55-64.	11.15 — 13.80	34.91 — 54.35
" Manihot (l) ..	" " **8** : 55-64.	13.27	35.55
Celastrus scandens	Harris, Rep. M. B. G. **20** : 122.	.....	31.42
Cytisus scoparius.....	Harris, Amer. Nat. **43** : 354.	.....	35.24
" "	Pearson, Phil. Trans. A. **197** : 335.	.....	36.78
Cercis canadensis.....	Harris, Bot. Gaz. **50** : 121.	19.26	26.71
Nelumbium luteum....	Pearl, Amer. Nat. **40** : 760.	.....	17.45
Lotus corniculatus....	Pearson, Phil. Trans. A. **197** : 367.	.....	46.38 — 52.14
Lathyrus odoratus.....	" " " " **197** : 367.	12.65 — 13.39	27.67 — 34.96
" sylvestris....	" " " " **197** : 367.	6.79	32.91
Vicia Faba	" " " " **197** : 367.	.....	33.50
Vicia hirsuta.....	" " " " **197** : 367.	30.88	38.32

In percentage variability of number of seeds per fruit, *Crinum* stands far above any of the considerable number of species given in this table. Only in *Staphylea trifolia*[5] have I found coefficients of variation at all comparable in magnitude. These two species agree in having very skew distributions of number of seeds per fruit, but differ widely in range and mean, the mean number of seeds per fruit in *Crinum* falling in the neighborhood of the maximum number in *Staphylea*. When a sufficiently wide series of these exceptional cases are gotten together, discussions of the underlying morphological and physiological factors to which their peculiarities are due can be profitably undertaken.

3. Variation in Seed Weight.

An investigation of the size of the exceedingly variable seeds of this form presents two serious difficulties. They are so irregular that weighing is the only feasible method

[5] For the data on which these statements are made see four papers: Biometrika **7** : 453–504. 1910. — Zeitschr. f. Ind. Abs.-u. Vererbungsl. **5** : 173–188. 1911.—Beih. Bot. Centralbl. **28** : 1–16. 1912. —Bot. Gaz. **53** : 204–218; 396–414. 1912.

of measurement. Only fully matured seeds should be weighed, but one fruit of an inflorescence may be quite mature, while others are apparently still in a developing condition.

The first difficulty demands only time and patience. The second may be fairly well met by choosing only fruits with well-ripened walls, though even here some of the smaller seeds seem immature.

In 1905, 333 seeds, taken quite at random, were weighed and seriated in units of half a gram range. The method of collection of another sample of 2,000 in 1906 is described under Section 7. Table VI gives the data.

TABLE VI.

Class	1905	1906	Class	1905	1906
1 = 0 — 0.50	22	219	10 = 4.51 — 5.00	10	39
2 = 0.51 — 1.00	31	396	11 = 5.01 — 5.50	5	27
3 = 1.01 — 1.50	57	352	12 = 5.51 — 6.00	6	26
4 = 1.51 — 2.00	33	290	13 = 6.01 — 6.50	6	29
5 = 2.01 — 2.50	45	212	14 = 6.51 — 7.00	5	13
6 = 2.51 — 3.00	40	147	15 = 7.01 — 7.50	2	5
7 = 3.01 — 3.50	20	106	16 = 7.51 — 8.00	1	6
8 = 3.51 — 4.00	26	76	17 = 8.01 — 8.50	2	1
9 = 4.01 — 4.50	22	55	18 = 8.51 — 9.00	....	1

The distribution of weight of seed, like that of the number of seeds per fruit, cannot by any stretch of the imagination be regarded as conforming to the "normal," Gaussian, or "Quetelet's" curve, which some biologists seem to regard as the biologically normal type. The skewness is very conspicuous. The great majority of the seeds remain very small—those attaining a large size are rare. To what extent this may be due to a competition of the numerous ovules for a supply of food material, which is inadequate to bring many seeds up to the large size which we think of as characteristic of the bulbiform seeds of the Amaryllidaceae, can only be determined by further investigation along lines indicated later.

For constants (in the half gram units as indicated in the tables) I find:

Mean Weight of Seed:—

1905.....5.565 ± .121
1906.....4.369 ± .045
Diff.....1.176 ± .129

Standard Deviation:—

1905.....3.282 ± .086
1906.....2.971 ± .032
Diff..... .311 ± .092

Coefficient of Variation:—

1905.....58.972 ± 2.007
1906.....67.697 ± 1.000
Diff..... 8.725 ± 2.242

Apparently, the two series differ significantly in mean weight of seed, those for 1905 being about half a gram heavier than those of 1906. This is not at all a surprising result, for the weight of a *Crinum* seed must depend, in large measure, upon availability of plastic material and water. The variability, as shown by the coefficient of variation, is very great. Perhaps the variability differs for the two years, but because of the skewness of distribution, the probable errors calculated by the ordinary formulæ may not give decisive tests.

The relative variabilities for seed weight, like those for number of seeds per pod, are high, but data for comparison from other species are rare. Johannsen[6] gives coefficients of variation of from 10.5 to 17.5 for "pure lines" of beans, and I have found generally comparable values for large series of *Phaseolus vulgaris*. I know of no other data.

4. The Relationship between the Size of the Inflorescence and its Capacity for maturing Fruits.

This problem has already been considered for immature

[6] Rep. Third Int. Conf. Genetics 109. 1907.

inflorescences of *Staphylea*[7] and for mature inflorescences of *Celastrus*.[8] The correlation between the number of rays per inflorescence and the number bearing a fertile fruit appears in the 1905 series, in Table VII, and for the 1906 series in Table VIII.

TABLE VII.
Fruits Matured.

Flowers Formed.	2	3	4	5	6	7	8	9	10	11	12	
6	....	3	3	3	....	....	....	....	....	....	....	9
7	....	2	2	4	1	1	....	....	....	....	....	10
8	2	4	8	4	6	2	1	....	....	....	....	27
9	....	4	3	2	8	6	1	....	....	....	....	24
10	....	2	3	8	6	4	5	1	....	....	....	29
11	1	3	4	3	5	1	3	....	1	1	....	22
12	....	1	6	2	3	3	5	1	....	....	....	21
13	....	....	3	1	3	3	3	3	....	....	....	16
14	....	3	4	4	3	2	4	1	....	....	1	22
15	....	....	....	....	2	2	1	....	2	....	....	7
16	....	....	....	....	1	3	....	....	....	....	....	4
17	....	....	....	....	....	....	....	1	1	....	....	2
	3	22	36	31	38	27	23	7	4	1	1	193

TABLE VIII.
Fruits Matured.

Flowers Formed.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
4	...	...	...	1	...	...	...	...	...	...	...	...	...	...	1
7	...	...	1	1	2	2	1	...	...	...	...	...	...	...	7
8	...	...	...	4	11	11	8	1	...	...	...	...	...	...	35
9	...	...	1	3	4	6	10	3	1	...	...	...	...	...	28
10	...	1	1	2	6	11	12	6	4	3	...	...	...	...	46
11	1	...	2	...	8	7	3	5	5	4	1	...	...	...	36
12	...	...	...	4	5	6	8	4	6	5	4	2	...	...	44
13	1	...	...	1	4	6	5	6	5	8	4	2	1	...	43
14	...	1	...	5	2	3	5	3	7	4	2	3	...	1	36
15	...	...	1	2	3	...	2	2	2	1	1	1	...	...	15
16	...	...	...	...	1	3	1	1	2	1	...	...	...	...	9
17	...	...	...	...	...	...	2	2	...	...	...	...	...	...	4
18	...	...	...	...	...	1	1	...	2	1	...	...	...	...	5
	2	2	6	23	46	56	58	33	34	27	12	8	1	1	309

[7] Harris, J. Arthur. Biometrika **6** : 439–441. 1909.
[8] Harris, J. Arthur. Ann. Rept. Mo. Bot. Gard. **20** : 117–119. 1909.

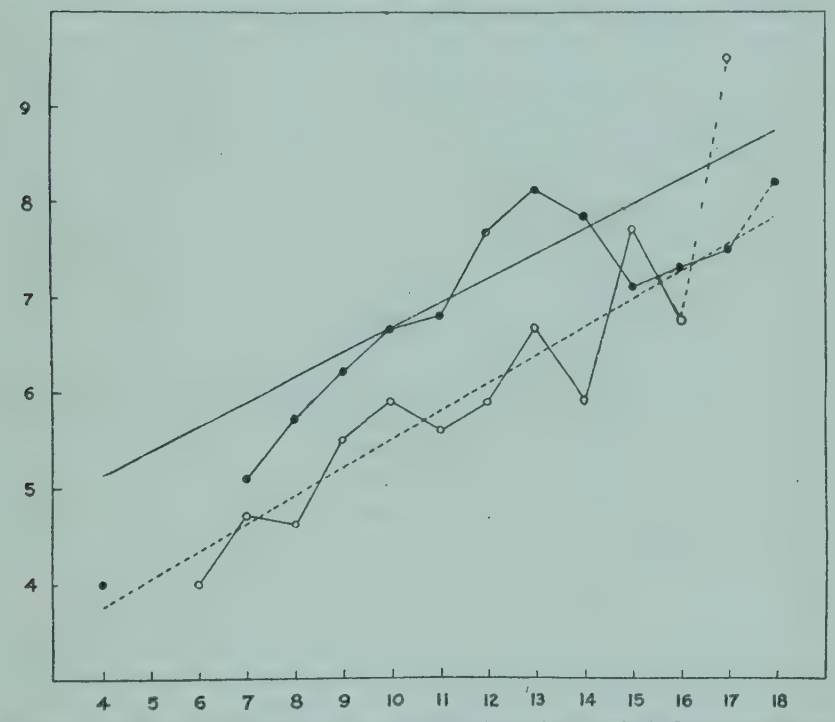
That the inflorescences with a number of flowers above the average produce more than the average number of fruits is clear from mere inspection of the tables. That the relationship is not a very close one is shown by the correlation coefficients for number of flowers and number of fruits which are:—

For 1905, $r_{ff} = .393 \pm .041$
For 1906, $r_{ff} = .305 \pm .035$
Difference $= .088 \pm .054$

Considering the smallness of the samples, the agreement is good.

The relationship between the two characters is shown graphically in Diagram 2, where the two lines are given by the straight line regression equations:—

DIAGRAM II.
REGRESSION OF NUMBER OF FRUITS MATURING ON NUMBER OF FLOWERS FORMED.



1905 series, circles and broken line; 1906 series, solid dots and firm line.

For 1905, $fr = 2.5912 + .2904\ fl.$
For 1906, $fr = 4.0703 + .2600\ fl.$

The lines are apparently a fairly good smoothing of the

empirical means—considering the smallness of the available samples. The means for 1906 perhaps suggest non-linear regression, but our data hardly justify further statistical treatment. The coefficient of correlation, r, may without further test be regarded as a *fairly* satisfactory measure of the relationship under consideration.

Here, as in the inflorescences already studied, we cannot be satisfied with the information given by the coefficient of correlation for number of flowers and number of fruits per inflorescence. The clusters with the larger number of flowers would be expected to produce more than the average number of fruits, providing that the chances of an individual flowers producing a fruit is not, in some measure, dependent upon the number of flowers in the inflorescence upon which it is borne.

The constant which we desire is r_{xz}, where x is to be read as number of flowers per inflorescence and z as the deviation of the number of fruits per inflorescence from their probable value, on the assumption that the chances of a flower producing a fruit is independent of the number of flowers per inflorescence.

Using the formula

$$r_{xz} = \frac{r_{xy} - V_x / V_y}{\sqrt{1 - r_{xy}^2 + (r_{xy} - V_x / V_y)^2}}$$

where x denotes number of flowers and y number of fruits, I find:—

For 1905, $r_{xz} = -.337 \pm .043$
For 1906, $r_{xz} = -.399 \pm .032$
Difference $= .062 \pm .053$

Again the agreement of the two series is as close as one could expect, having regard to the probable errors. The signs show that as the number of rays per inflorescence increases, the capacity of the individual rays for maturing their ovules into seeds decreases, and to a considerable extent.

The other two cases, which have been investigated give:—

For *Staphylea*, $r_{xz} = -.649 \pm .024$
For *Celastrus*, $r_{xz} = +.024 \pm .034$

The *Staphylea* material was a lot of immature inflorescences in which further failures would have taken place before maturity. The *Celastrus* series shows that the results obtained for *Crinum* cannot be regarded as general. Only the investigation of many further series of data will enable one to speak with confidence concerning the relationship of the size of the inflorescence to its capacity for maturing its ovaries into fruits, or to discuss the factors involved. The constants already calculated suggest that the problem is one of real physiological interest.

5. The Relationship between the Number of Fruits per Inflorescence and the Number of Seeds produced.

A first step in ascertaining the factors which are active in determining the fertility of a fruit may be taken in the calculation of the correlation between the number of fruits per inflorescence and the number of seeds which these fruits contain.[9] If the development of an abnormally large number of fruits per inflorescence makes severe demands upon the plastic materials which can be supplied to it, then, one might expect a negative correlation between the number of fruits matured and the number of seeds developing per fruit. On the other hand, the greater number of fruits on some inflorescences may merely indicate superior vigor, or feeding; in this case, the number of seeds per fruit might tend to increase as the number of fruits per inflorescence becomes larger. Doubtless both are active factors and must ultimately be analyzed out and measured individually. Doubtless the two factors tend, in many cases, to cancel each other, giving a resultant correla-

[9] This has been done for an extensive series of fruits of *Staphylea*. See two papers, one in the Botanical Gazette **53**: 204–218; 396–414. 1912; and another in the Beihefte zum Botanischen Centralblatt I. **28**: 1–16. 1912.

tion of *circa* 0. Preliminarily, I have thought it worth while to determine in a few cases what the actual correlations are. Such results must be of service in planning more detailed and critical studies.

Unfortunately, only the small series of inflorescences for 1905 is available for our present purposes.[10] The correlation between number of fruits per inflorescence and number of seeds per fruit (fruits per inflorescence weighted with the number of seeds which they produce) is shown with a 5-unit grouping for the seeds in Table IX.[11] The constant is

$$r = -.072 \pm .024^{12}$$

TABLE IX.

SEEDS PER FRUIT.

Fruits per Inflorescence	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55	
2	1	....	.....	1	2	.....	1	.....	.....	.....	1	6
3	24	11	8	7	6	6	2	1	1	.....	.....	66
4	64	22	8	14	11	2	3	1	2	1	.....	128
5	68	18	7	6	6	2	1	2	.....	.....	.....	110
6	65	26	16	7	12	6	2	2	1	.....	1	138
7	76	23	16	11	5	.....	1	.....	1	.....	.....	133
8	64	17	12	6	5	8	2	2	2	.....	2	120
9	13	11	7	1	.....	2	.....	1	1	.....	.....	36
10	9	6	1	1	1	.....	.....	.....	1	.....	1	20
11	8	2	1	.....	.....	.....	.....	.....	.....	.....	.....	11
	392	136	76	54	48	26	12	9	9	1	5	768

Apparently, therefore, the production of a number of fruits

[10] These were only 193 in number and 50 had one or more of the fruits dehisced, so that the number of seeds in them could not be obtained. These fruits probably represent, on the average, those containing the greatest number of seeds, since these ripen first. It seemed best, therefore, to discard all inflorescences in which dehisced fruits were found.

[11] Probably grouping by fives is too coarse, where variation is as skew as it is here. In another study this point should be regarded.

[12] This was obtained by using 768, the number of fruits, as N. If the number of inflorescences, $N = 143$, be used, we have $r = -.072 \pm .056$

above the average tends to cut down the number of seeds in these fruits,[13] but much confirmatory evidence must be obtained before great stress can be laid on such a weak correlation.

The only directly comparable series are one for *Celastrus*[14] and several for *Staphylea*.[15] For *Celastrus* there is no trustworthy indication of an interdependence between the number of flowers formed per inflorescence and the number of seeds maturing per fruit, or between the number of fruits matured per inflorescence and the number of seeds matured per fruit.

For *Staphylea* the results show the most slender relationships between numbers of fruits per inflorescence and the fertility of the fruits. Data for both ovules and seeds are available. For twenty individual shrubs of 1906, the mean relationship for number of fruits per inflorescence and number of ovules per locule is only $r = +.0192 \pm .0185$ —a value of no significance. For fruits and seeds it is $r = -.0399 \pm .0080$, indicating that in *Staphylea* the condition may be similar to that in *Crinum*. For the whole populations the results are:—

	For Ovules.	For Seeds.
1906,	$r = +.0391 \pm .0086$	$r = -.0474 \pm .0086$
1908,	$r = +.0633 \pm .0061$	$r = -.0494 \pm .0061$
1909,	$r = -.0539 \pm .0085$	$r = +.0626 \pm .0085$

Taken as a whole, the data for *Staphylea* indicate a very slight negative correlation between the number of fruits matured and the capacity of these fruits for developing their ovules into seeds.

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[13] Waldron (Am. Nat. **44** : 48–56. 1910.) concludes that the seeds are lighter in large heads of oats than in small ones. Harris (Torreya **11** : 165–169. 1911.) has shown that in *Staphylea* and *Cladrastis*, the weight of the seed is in some measure dependent upon the number produced in the fruit. As noted above, other data are in press, and still more are being reduced.

[14] Harris, J. Arthur. Ann. Rept. Mo. Bot. Gard. **20** : 116–122. 1909.

[15] Harris, J. Arthur. Beih. Bot. Centralbl. I. **28** : 2–10. 1912.

But altogether the evidence confirms the conclusions from another study[16] that the correlation between somatic characters and fertility is generally very slight.

6. Correlation in Seed Production between the Fruits of the Inflorescence.

If the number of seeds which develop per fruit is dependent upon the available plastic material, as seems somewhat probable from the results of the two preceding sections, then one might expect an intra-inflorescence correlation for number of seeds per fruit. That is, one would expect that the characters of an individual fruit—judged in comparison with the condition in the population as a whole as a standard—would not be independent of, but correlated with, those of the other fruits of the inflorescence.

The great difficulty of the problem lies in its complexity. Within a given inflorescence an abnormally large number of seeds in one fruit might necessitate an abnormally small number in another fruit, because of the inadequacy of plastic materials. But the available materials may differ widely from inflorescence to inflorescence, and as a consequence, all of the fruits of some inflorescences be much better supplied than those of others. The tendency of the first of these putative conditions would be to produce a negative correlation between the number of seeds per fruit of the inflorescence, while that of the second would be to bring about a positive correlation. As yet, I see no way of differentiating these factors and estimating their relative intensities. Certainly, this is not possible in the present material. It seems worth while, however, to make a beginning in the problem by determining what the actually existing correlation is.[17]

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[16] Harris, J. Arthur. On the Correlation between Somatic Characters and Fertility. Biometrika **8** : 52–65. 1911.

[17] This problem is being considered in some detail for a widely different species. See a paper, The Influence of the Seed upon the Size of the Fruit in *Staphylea*, in the Botanical Gazette **53** : 204–218; 396–414. 1912.

From the material collected in the spring of 1906, it is possible to form correlation tables for the number of seeds per fruit in the same inflorescence.

A difficulty at once arises in regard to the selection of fruits to represent the inflorescences. The complete failure of some flowers to produce seeds may, perhaps, affect the number which develop in other ovaries of that inflorescence. It is difficult to decide in advance whether all pedicels shall be chosen quite at random and the sterile ones inserted in the series, or whether all pedicels bearing no fruits shall be excluded.

From the 1906 lot of material, it is possible to extract two series. The first comprises 249 inflorescences bearing five fruits each. It would have been slightly better, perhaps, if all the fruits from these inflorescences had been used, but the labor of dealing with the correlations is excessive. The second series comprises 280 inflorescences each yielding eight pedicels with or without fruits.[18] In both of these series the pedicels or fruits were chosen quite at chance by a system of numbering the recorded data backwards and forwards.

The tabling of the data presented a problem of the greatest difficulty. As compared with many symmetrical intra-class correlation surfaces which have been formed, the number of entries is small, being only $5 \times 4 \times 249 = 4{,}980$ for the table containing fertile fruits only and $8 \times 7 \times 280 = 15{,}680$ for the surface comprising both sterile and fertile rays. But the range of variation in number of seeds is so great—from 0 to 70—that the tables are exceedingly large, comprising 4,900 and 5,041 compartments respectively. It is not safe to reduce the surface of the table by grouping, because of the skewness of distribution.

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[18] Sterile fruits were counted in with those in which the fruit failed to develop, since the persistence of the ovary, when no seeds develop, cannot be regarded as forming a separate category as far as our present considerations are concerned. Our pedicels are classified according to the number of seeds which they produce, hence "no fruit" and "sterile fruit" both fall in the *zero* class.

The table of 4,980 entries was successfully formed and verified; that of 15,680 entries presented so many difficulties that it was finally given up in despair. Fortunately, just at the time, a relatively short process of obtaining the intra-class correlation coefficient from untabulated data was successfully worked out.

The constants published here were, therefore, obtained by two different but equally valid methods, which applied to the same material would give identical results.[19] We find for the two series:—

For fertile fruits only, $r = +.1556 \pm .0187$[20]
For sterile and fertile rays, $r = -.2149 \pm .0136$

Now these constants must be cautiously interpreted, but looking at them superficially—which is all that is possible until much more extensive and refined data are available—one is inclined to conclude *(a)* that the inflorescences vary[21] in (α) the opportunities for pollination, (β) the innate vigor (capacity for development) of the ovules, or (γ) the quantity of plastic material available for seed formation, so that when one fertile fruit has a high number of seeds, the others also tend to be high, but *(b)* that an excess of fertility in some fruits of an inflorescence is in the long run attained at the cost of the complete sterilization of others.

But these conclusions are purely tentative—chiefly useful as indicators of fertile lines of future research into a complex subject.

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[19] The new method will be published shortly. The original table in the one case and the untabulated data in the other are too bulky for publication. The arithmetical routine has been carried out with exceeding care, and checked at every step.

[20] I follow the general practise of taking N as the actual number of fruits in the calculation of the probable error. Possibly, we should use the number of inflorescences instead. In that case, we would have: $r = +.1556 \pm .0427$, and $r = -.2149 \pm .0384$.

[21] The reader will bear in mind that these inflorescences come from a large number of plants. Hence, differences shown by inflorescences are partly, at least, the differences between individuals.

7. Correlation in Weight between the Seeds of the Fruit.

In the foregoing paragraphs, an attempt has been made to learn something of the factors influencing the number of seeds per pod. The original plan was to make these studies much wider and more detailed, and to investigate the factors influencing seed weight. The only question which can be investigated on the preliminary records is whether a difference in the capacity for seed development is to be detected in the size of the seeds from various fruits.

Of the various ways in which this problem might be approached, the most satisfactory seems to be to determine the degree of similarity in weight of seeds from the same fruit. This procedure is justified by the nature of the problem. If the *Anlagen* giving rise to some fruits are more vigorous than those giving rise to others, or the fruits better supplied with plastic materials, we might expect the seeds to be heavier, while in the less vigorous or less richly nourished fruits, they would be lighter. These factors would, to some extent, affect all of the seeds of a fruit alike, and as a result, a correlation between the weight of the seeds of the same fruit would arise. The magnitude of the intra-ovarial correlation coefficient measures (with certain restrictions)

TABLE X.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1	918	577	166	87	70	57	26	23	19	8	12	4	3	1	..	..	..	..	1,971
2	577	1,510	748	361	179	91	39	21	16	9	2	5	2	3	1	..	..	..	3,564
3	166	748	962	644	327	157	98	32	15	11	3	2	2	...	..	1	..	..	3,168
4	87	361	644	576	451	233	133	41	44	19	8	4	5	2	..	2	..	..	2,610
5	70	179	327	451	360	213	134	66	43	29	17	8	7	2	1	1	..	..	1,908
6	57	91	157	233	213	174	153	92	56	39	18	14	17	5	..	4	..	..	1,323
7	26	39	98	133	134	153	98	94	58	54	17	20	20	7	2	1	..	..	954
8	23	21	32	41	66	92	94	82	67	61	31	18	29	17	2	8	..	..	684
9	19	16	15	44	43	56	58	67	30	33	25	25	34	16	5	7	1	1	495
10	8	9	11	19	29	39	54	61	33	22	18	18	15	4	3	6	..	2	351
11	12	2	3	8	17	18	17	31	25	18	32	11	28	10	3	5	3	..	243
12	4	5	2	4	8	14	20	18	25	18	11	30	39	15	12	5	1	3	234
13	3	2	2	5	7	17	20	29	34	15	28	39	20	19	9	8	2	2	261
14	1	3	...	2	2	5	7	17	16	4	10	15	19	6	5	4	1	..	117
15	...	1	...	...	1	...	2	2	5	3	3	12	9	5	..	..	1	1	45
16	...	...	1	2	1	4	1	8	7	6	5	5	8	4	..	2	..	..	54
17	...	...	...	...	...	...	...	...	1	...	3	1	2	1	1	..	..	..	9
18	...	...	...	...	...	...	...	...	1	2	...	3	2	...	1	..	..	..	9
	1,971	3,564	3,168	2,610	1,908	1,323	954	684	495	351	243	234	261	117	45	54	9	9	18,000

the degree of individuality of the fruits with respect to their capacity for laying down seed-forming substance.

The 1906 series of weighings were made for this purpose. The labor involved in the preparation and verification of correlation tables with every seed used once as a first and once as a second member of an associated pair is very greatly increased by the use of varying numbers from the fruits. It is desirable, therefore, to have the same and as large a number as possible from each of the 200 fruits. Ten can easily be secured from the larger earlier maturing fruits. The fruits, therefore, are not a random sample of the whole crop, but are intentionally a selection, though only in so far as is necessary to secure those containing at least ten seeds. The seeds to be weighed were chosen quite at random by selecting those falling farthest to the right when the fruit was opened above a table.

The symmetrical intra-fruit correlation surface appears as Table X.[22] Within each fruit there are $n(n\text{-}1)$ combinations, or a total of $10 \times 9 \times 200 = 18{,}000$. The correlation coefficient is

$$r = .6759 \pm .0082$$

and the equation to the regression straight line, shown graphically in Diagram III is

$$W_2 = 1.4225 + .6759\, W_1,$$

where the constants are in the half-gram units of weighing. The close agreement of the empirical and theoretical means leaves no question concerning the linearity of regression.

The results indicate that some complex of morphogenetic and physiological factors tends very strongly to make the seeds of a fruit alike in weight. The nature of these factors and the part played by each must be determined by the application of proper biometric formulæ to more detailed records.

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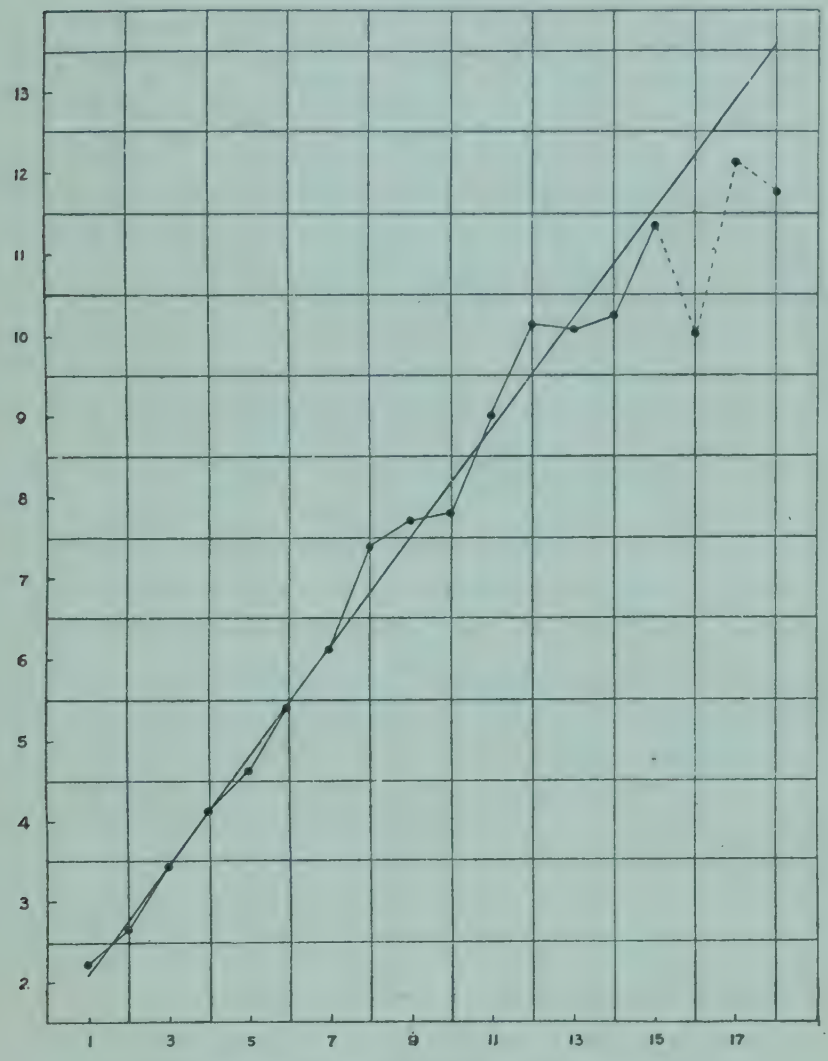
22 The seed weights are for convenience expressed in the class units given in Table VI.

DIAGRAM III.

EMPIRICAL MEANS AND FITTED REGRESSION STRAIGHT LINE, SHOWING CLOSENESS OF INTERDEPENDENCE IN WEIGHT OF SEEDS FROM THE SAME FRUIT.



IV. SUMMARY AND CONCLUSIONS.

1. The primary purpose of this paper is the recording of quantitative data on the inflorescence, fruit and seed of *Crinum longifolium* for future use in comparative studies of fertility and fecundity in plants. Such problems are immensely complex. Numerous forces are pulling, sometimes in the same, sometimes in opposite directions. Another investigator may find his material differing in some important regards from that described here, because various

conditions are different. It is, therefore, only with the explicit statement that final conclusions must await the comparative treatment of wider series of data, that I indicate some of the points of more general interest.

2. On comparative grounds, one must assume that the anomalous seed habit of *C. longifolium,* and other similar species, is not primitive but probably recently acquired from an ancestral form producing a large number of ovules per fruit. Several peculiarities of the inflorescence and fruits may be referred to the large size of the seed. We note that:—

(a) Variation in the number of flowers produced or in the number of fruits matured per inflorescence is not greater than that generally found in inflorescences. The peculiarities of the seeds apparently have not produced any effect on variation in the inflorescence.

(b) The variation in number of seeds per fruit, measured by range, standard deviation or coefficient of variation is very great. The distribution is also very skew. These conditions are probably directly due to the existence of a large number of ovules in each ovary (an ancestral characteristic?) of which only a part can, because of the great size of the seeds, be developed to maturity.

(c) The distribution of seed weight is very skew and the variability very high. This is probably to be attributed to the limitation imposed upon the tendency of a large number of ovules to develop into seeds by the inadequacy of plastic materials for all.

3. There is a moderately close positive correlation of the order $r = .35$, between the absolute numbers of flowers formed and fruits developing per inflorescence. The correlation between the number of flowers per inflorescence and the deviation of the number of fruits developing from the probable, on the assumption of proportionate fertility throughout, is negative in sign and of about the same order of magnitude. The larger inflorescences are, therefore, less

capable of maturing their ovaries into fruits than are the smaller ones.

4. There appears to be a slight negative correlation between the number of fruits per inflorescence and the number of seeds developing per fruit, *i. e.,* a decrease in the number of seeds per fruit is associated with the production of a number of fruits above the average. This result reinforces the conclusions stated under (3).

5. The intra-inflorescence correlation for number of seeds matured is positive if only fertile fruits be included, but (apparently) significantly negative, if sterile and fertile ovaries are taken at random. Apparently, therefore: *(a)* the inflorescences vary in their capacity for forming seeds, so that when one fruit is above the average in seed production, the others of the same inflorescence are also likely to be above the average in fertility; *(b)* the superior fertility (seed production) of some fruits is likely to be attained at the cost of the complete sterilization of other ovaries.

6. There is a moderately high correlation between the weight of the seeds of a fruit. There is, therefore, some complex of factors—innate vigor of ovules, availability of plastic materials, etc.,—tending to render the seeds of a fruit alike. What these factors and their intensities are, can only be ascertained by more detailed analysis of more extensive data.

THE RELATION BETWEEN THE DENSITY OF CELL SAPS AND THE FREEZING POINTS OF LEAVES.[1]

WILLIAM WOODWARD OHLWEILER.

INTRODUCTION.

The optimum temperatures for plant growth vary greatly for different regions, and the ranges of variation for different plants are almost as varied as the plants themselves. Where plants are grown under glass it is necessary to vary the temperature according to the requirements of the plants to be grown, as is shown by the use of tropical, subtropical, stove, temperate, and cool houses. Some plants are closely limited to these temperatures, while others seem to be more or less cosmopolitan. However, for the species and varieties of plants native to any one region, there is this same variation in the temperature requirements, some seeking the cool, wet bogs, and others the dry, sunny slopes. We have, in any region, plants that are very susceptible to cold periods, and others that are not. Late spring frosts destroy some crops with apparent regularity, while others remain unharmed. Why is there this difference? It could hardly be hereditary, since long years of acclimatization would have eliminated the weaklings. The structure of the cell walls might determine whether or not cells of certain plants would freeze. However, it is not the freezing of the cell wall which kills plants, but the freezing of the cell contents. It is more or less true that the cell walls may have some effect in retarding or preventing the freezing of the contents, but this, when we consider the small amount of tissue involved, would have but little influence upon the

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1 A thesis presented to the Faculty of Washington University in candidacy for the degree of Master of Arts, June, 1911.

final result. Can we then narrow our argument down to the ability of the cell sap to resist freezing?

In the latter part of April, 1910, there occurred throughout the State of Missouri, a drop in temperature to 25°F. Foliar growth of the trees and shrubs was in an advanced condition, and the freeze, which was accompanied by rain and snow, did a great deal of damage to all green material, but, as is the case generally, destroyed the foliage of some trees and shrubs more than of others. As a matter of personal interest, the damage to foliage was recorded in detail, and these notes occur in this paper as Table 1. From these observations alone, no conclusions as to the reason for the difference in resistance to freezing were apparent. Many plants which would reasonably have been expected to behave the same, acted quite differently, and the problem became more perplexing the more it was studied.

A year later, *i. e.,* in the spring of 1911, it was decided to extract the saps of the various leaves, freeze them artificially, and determine their freezing points, thereby ascertaining if any correlation existed between this artificial behavior and that actually exhibited in the freeze of the year before. These results and conclusions are the basis of this paper. Only trees and shrubs growing in the Missouri Botanical Garden, out of doors, were observed, and their native habitats, *fide* Index Kewensis, are given in Table 1. As a matter of convenience it has seemed advisable to separate the results of natural from artificial freezing, the two being essentially different investigations, and to summarize in the conclusion such correlations as may occur.

The paper then comprises:—

1. Notes on the effect of the freeze of April 24, 1910, on the trees and shrubs growing in the Missouri Botanical Garden, their recovery and other data.

2. The freezing point of leaf saps, determined artificially, and notes in connection therewith.

3. Conclusions.

1. NOTES ON THE EFFECT OF THE FREEZE OF APRIL 24, 1910, ON THE TREES AND SHRUBS GROWING IN THE MISSOURI BOTANICAL GARDEN, THEIR RECOVERY AND OTHER DATA.

The continental position of Saint Louis is such, that it is practically unaffected by the proximity of large bodies of water or mountain ranges. It lies in a large river valley about midway between the divides of the Appalachian and Rocky Mountain ranges. In every direction the country is open for hundreds of miles, except as it is interrupted by the retaining walls of the two mountain ranges already referred to. To the south lies the Gulf of Mexico, and it is, as might be expected, one of the storm-forming areas of this section. The storms from this section are in the main warm, and although often destructive, need not be considered in connection with this paper. To the north the storm area is more varied, and the storms that sweep down from this section are usually very cold, and often very sudden. Since east and west need not be taken into consideration as affecting temperature changes directly, they may indirectly be responsible for subjecting Saint Louis to the extremes of temperature from the north and south, *i. e.,* they form the channel through which these winds are forced to blow. Since it is unaffected by the tempering influence of large bodies of water, its frost period is much later than that of cities of corresponding latitude on the oceans, or near them. (Dorschied, Metr. Zeitschr. **24**: 11-24. *f. 1.;* 49-64. *pl. 1.* 1907.) Dorschied places Saint Louis on the same line, as regards beginning, ending and mean duration of frosts, as points on the coast of Alaska, British Columbia, New England, Southern Iceland, Norway, and the Western Alpine and Mediterranean regions. The average date of the last killing frost for Saint Louis is April 2. (Garriott, E. B. Notes on Frost. Farmers' Bull. U. S. Dept. Agr. 104: 16. 1910.) The prevailing direction of the wind during the month of March, 1910, was from the southwest, and means that this was a warmer month than usual. Quoting the summaries for March and

April (U. S. Dept. Agr., Weather Bur., Monthly Metr. Summary for Saint Louis, Mo., March and April, 1910):—

"The weather for March was very unusual. The mean temperature was 57.5°, which is 3.2° higher than for any previous March, and the temperature was continuously above the normal, except on the 9th, 10th, 14th, and 15th. The maximum temperature for the month was 87, and this has been exceeded but once in March in the history of the station. Freezing temperatures occurred on three days only. The precipitation was the lowest ever recorded for March, and only twice in forty years has a smaller amount been recorded for any month. The number of clear days, 22, is the highest ever observed in March, and the number of cloudy days, 2, the lowest. The sunshine was 79 per cent of the possible amount, and was the greatest for March since the records began. During the latter part of the month the atmosphere was very hazy, causing the sun and the moon to appear very red. The wind movement, while not the least for any March, was much below the average, and a less amount has been recorded five times only in March."

and for April:—

"The temperature for April averaged slightly below the normal and was characterized by great extremes, the maximum being surpassed but once in the history of the station. The minimum, while not the least for any April, was unusual for the last decade of the month. All previous low temperatures have been recorded in the first half of the month. The minimum of 25° on the 24th was the lowest minimum after the 20th in 74 years. The low temperature was caused by a slow movement of a storm area from Illinois to lower Michigan that developed in intensity and brought down cold air from the northwest. The mean temperature for April was 1.7° lower than that of March. A similar case occurred in April, 1907, when the mean temperature for April was 5.9° lower than March. The snowfall was the heaviest for any April, but never before has so great an amount fallen as late as the 24th of the month."

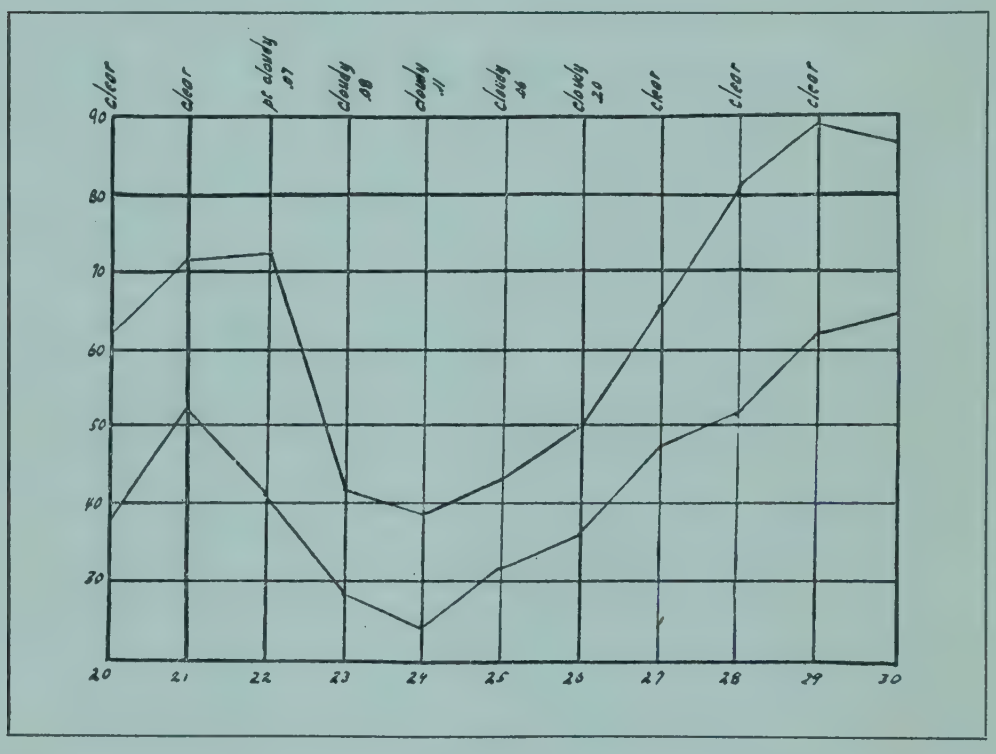
The location of the Garden on a high point of land, with well-drained areas sloping toward the west and northwest, makes damage from the average frost not a very common occurrence. Ground frost and consequent damage to tender herbaceous plants sometimes occurs, but often when frost has been known to occur on the ground, there has not been any noticeable effect on the leaves or flowers of trees and shrubs. The Garden is also slightly favored in

an ordinary frost by the fact that the major part of the slope is north and northwest. However, "the cold spell of April 24, 1910, was accompanied by cloudy skies and rain, mixed with snow, and the movement of the cold air currents was quite different from that which takes place under a clear sky, or when there is no rain or snow. It will be observed that during cold spells, with more or less cloudiness or moisture, the temperature on the hills may fall to a lower value than the temperature in the valleys. On the other hand, had clear weather prevailed * * * the temperature conditions would have been reversed, the valleys probably being colder." (Garriott, E. B. Notes on Frost. Farmers' Bull. U. S. Dept. Agr. 104: 12. 1910.) Moreover, the minimum temperature of the 24th was, as reported by the Weather Bureau, 25°F., and acted for a time long enough to insure freezing of such things as were in condition to freeze. As a matter of fact no frost was formed, and the results are simply those of direct freezing. This makes the later comparisons of natural and artificial freezing much more convincing than would be the case had the damage to vegetation been due to frost, for we could not be quite sure that we had duplicated frost conditions of temperature and moisture, while duplicating freezing temperatures is a comparatively easy matter.

The effect of cold upon vegetation in general depends very largely upon the rapidity with which the destructive change in temperature is brought about, being greater when the change takes place within narrow limits of time. Temperature changes in March cannot be said to directly concern the plants affected by a freeze during the next month, yet the same temperatures occurring a few days before a heavy freeze, could have serious results. In the one case acclimatization results, whereas in the other no adaptive change takes place. In how far the process of acclimatization can protect plants from extremes of cold we do not know, although the principle is applied in using cold frames to acclimatize former greenhouse plants. How-

ever, the case under consideration represents the reverse process, since the weather to which these plants were being subjected was gradually adapting them to warmer instead of colder weather. It would seem then, on the face of things, that frosts occurring in the spring find the plants, as a rule, preparing for the growing season and warmer weather, while those occurring in the fall and at the close of the growing season, find the plants tending to acclimatize themselves to the approaching cold weather. There should be no doubt, then, as to the period of the year in which a frost is the more destructive.

The immediate condition of the weather around the 24th of April, *i. e.*, the period from the 20th to the 29th deserves some attention, and the following temperatures, maximum and minimum, are here given, and are well illustrated by the appended diagram:—



in which the ordinates are the temperatures recorded for the ten days represented by the abscissae. The weather for

each day is recorded at the upper edge of the diagram, and the precipitation, if any, underneath the word "cloudy," "partly cloudy," etc., the precipitation for clear days being zero. The upper line is for the maximum, and the lower for the minimum temperature. It will be seen from the diagram that during a period of clear weather the temperature gradually rose until reaching the 72° mark, dropping from here to 28° the next night, and to the minimum of 25° the following night—the greatest drop for any 24-hour period being 44°. It is quite probable that the drop just referred to, was the one that did the greater part of the damage, although it is quite certain that the next two days of freezing, with the minimum at 25°, could leave but little to be desired for a good freezing test. Furthermore, the extent of the freeze was quite general over the entire state, and from no section was complete immunity recorded.

Foliar conditions in the main paralleled those of the weather. During the month of March conditions, except those of moisture, were very favorable for foliage growth. In spite of this, foliar growth was far in advance of the season. It would be difficult to show specifically the progress that vegetation had made, for it would be easier to tell what had not as yet started, but even this would not be possible, because all trees had leafed out somewhat. The stage of leafing is, then, merely one of degree. *Syringa vulgaris* had flowered, while at the same time *Catalpa* was still in the cluster or bunched condition, while *Sambucus canadensis* must have barely started. However, for determining the identical stages of growth in subsequent years the leaves of the Norway maple and the stage of growth of the *Syringa* may be taken as standards. Both of these were in full leaf.

The foliage of the trees immediately after the freeze presented an appearance that suggested fire. The northwest side of the trees was badly frozen in all cases, even on the evidently hardy material. Blackened, shapeless leaves, which

TABLE I.

Name of Plant	Habitat	Condition at the Time of Frost			Condition Several Days Later			Adventive Buds	Growth Two Weeks Later
		In Leaf	In Flower	In Bud	Effect on Leaf	Effect on new Shoot	Effect on Bud		
1. Vitis vinifera	Or., N. W. Ind.	Y	Y	?	K	K	K	G	N
2. Morus alba	Temp. As.	Y	Y	Y	K	PK	PK	G	N
3. Magnolia acuminata	N. Amer.	Y	Y	N	K	N	N	NG	Fair
4. Magnolia Yulan	China	Y	Past	N	K	N	N	N	Started
5. Liriodendron tulipifera	N. Amer. & China	Y	?	Y	K	PK	PK	G	Fair
6. Catalpa bignonioides	N. Amer.	Y	N	P	K	K	K	G	Slow
7. Fagus sylvatica purpurea	Europe	P	N	P	PK	N	N	N	Good
8. Aesculus Hippocastanum	As. Min.	Y	Y	N	PK	N	PK	N	Good
9. Ostrya virginica	N. Amer.	Y	N	N	PK	N	N	N	Good
10. Ginkgo biloba	Japan	Y	N	N	K	N	N	N	N
11. Fraxinus Ornus	Med. Reg., Or.	Y	N	N	K	PK	N	G	Fair
12. Rhus Toxicodendron	N. Amer.	Y	N	N	K	K	N	G	None
13. Koelreuteria paniculata	China	Y	N	Y	K	N	N	G	N
14. Cercis canadensis	N. Amer.	Y	Past	N	K	N	N	G	N
15. Cladrastis tinctoria	N. Amer.	Y	N	N	K	N	N	G	N
16. Hydrangea quercifolia	N. Amer.	Y	N	N	K	N	N	G	G
17. Fraxinus americana	N. Amer.	Y	N	Y	K	PK	N	G	N
18. Acer dasycarpum	N. Amer.	Y	Past	N	PK-N	N	N	N	G
19. Acer platynoides	Eu., Or.	Y	Past	N	PK-N	N	N	N	G
20. Gleditschia triacanthos	N. Amer.	Y	N	Y	K	PK	N	G	Slow
21. Paulownia imperialis	Japan	Y	N	Y	K	K	K	G	N
22. Clethra alnifolia	N. Amer.	N	N	N	N	N	N	N	Started
23. Ulmus campestris	Eu., Or.	Y	N	N	PK	N	N	N	G
24. Magnolia obovata discolor	Japan	Y	Y	Y	PK	N	N	N	Fair
25. Magnolia glauca	N. Amer.	P	N	Y	PK	N	N	N	Fair
26. Viburnum Opulus sterilis	Eu., N. As., Amer.	Y	Y	N	N	N	N	N	Good
27. Cornus Mas	Eu., As. Min.	Y	N	N	N	N	N	N	Good

TABLE I—Continued

Name of Plant	Habitat	Condition at the Time of Frost			Condition Several Days Later			Adventive Buds	Growth Two Weeks Later
		In Leaf	In Flower	In Bud	Effect on Leaf	Effect on new Shoot	Effect on Bud		
28. Rhus Cotinus atropurpureus	Med. Reg., Him. Reg., Or., China	Y	N	N	K	K	N	G	Slow
29. Chionanthus virginica	N. Amer.	Y	Y	N	K	K	N	G	Slow
30. Neillia opulifolia	N. Amer.	Y	N	N	PK	N	N	N	Good
31. Magnolia cordata	N. Amer.	P	N	Y	PK	N	N	N	Slow
32. Pinus Laricio (P. Austriaca)	S. Eu.	Old	N	Y	N	N	PK	N	Good
33. Pyrus arbutifolia erythrocarpa	N. Amer.	Y	Y	N	PK	N	N	N	Good
34. Aralia spinosa	N. Amer.	Y	N	Y	K	PK	N	G	Start
35. Platanus occidentalis	N. Amer.	Y	N	YP	PK	N	N	N	Slow
36. Quercus imbricaria	N. Amer.	Y	N	N	N	N	N	N	Good
37. Hydrangea paniculata	Japan	Y	N	N	PK	N	N	N	Good
38. Rhus typhina	N. Amer.	Y	N	Y	K	PK	N	G	Slow
39. Securingea fluggeoides	Japan	Y	N	N	K	PK	N	N	N
40. Taxodium distichum	N. Amer.	Y	N	N	K	?	N	N	N
41. Viburnum Lentago	N. Amer.	Y	Y	N	PK	N	Fls. PK	N	Good
42. Rhus copallina	N. Amer.	N	N	Y	N	N	N	N	N
43. Euonymus Sieboldiana	Japan	Y	Y	N	N	N	N	N	Good
44. Berberis vulgaris Thunbergii	Japan	Y	N	N	PK	PK	N	N	Fair
45. Rhamnus tinctoria	E. Eu., As. Min.	Y	N	N	PK	PK	N	N	Good
46. Spiraea van Houttei	Gardens	Y	Y	N	PK	Rarely	PK	N	Fair
47. Vitis hederacea (Ampelopsis quinquefolia)	N. Amer.	Y	N	Y	K	PK	N	N	N
48. Ilex quercifolia (I. opaca)	N. Amer.	Y	N	N	K	K	N	N	N
49. Magnolia Soulangeana	Gardens	Y	Past	Y	PK	N	N	N	Started
50. Lindera Benzoin	N. Amer.	Y	Y	N	PK	PK	N	N	Started
51. Magnolia Kobus	Japan	Y	N	N	N	N	N	N	Fine

TABLE II

Name of Plant	Habitat	Condition at the Time of Frost			Condition Several Days Later			Adventive Buds	Growth Two Weeks Later
		In Leaf	In Flower	In Bud	Effect on Leaf	Effect on new Shoot	Effect on Bud		
52. Ribes aureum	W. N. Amer.	Y	N	N	PK	N	N	N	Fine
53. Hamamelis virginiana	N. Amer.	Y	N	Y	PK	N	N	N	Slow
54. Philadelphus coronarius	S. Eu.	Y	N	N	PK	N	N	N	Fair
55. Philadelphus grandiflorus	N. Amer.	Y	N	N	PK	N	N	N	Fair
56. Halesia tetraptera	N. Amer.	Y	Past	N	PK	N	N	N	Fair
57. Diospyros virginiana	N. Amer.	Y	Past	N	K	K	N	G	N
58. Sassafras officinale	N. Amer.	Y	Past	N	K	K	N	G	N
59. Ailanthus glandulosa	China	Y	N	N	K	PK	N	G	N
60. Quercus tomentosa	Mexico	Y	N	N	PK	PK	N	Fair	Start
61. Fraxinus viridis	N. Amer.	Y	N	N	K	PK	N	G	N
62. Pyrus japonica	Japan	Y	Past	N	PK	N	N	N	G
63. Spiraea prunifolia	Japan	Y	N	N	PK	PK	N	N	G
64. Tsuga canadensis	N. Amer.	Y	N	N	K	K	N	N	G
65. Magnolia stellata	Japan	Y	Past	N	N	N	N	N	G
66. Taxus baccata	N. Temp. Reg.	Y	N	Y	K	K	N	G	Start
67. Diervilla rosea	China	Y	Y	N	N	N	N	N	Fine
68. Rosa rugosa	Japan	Y	N	Y	K	K	N	NG	Fair
69. Acanthopanax ricinifolium	Japan	Y	N	Y	K	N	N	N	Start
70. Xanthoceras sorbifolia	China	Y	N	N	K	N	N	N	Fair
71. Ligustrum amurense	Amur. Reg.	Y	N	N	PK	N	N	N	Good
72. Aesculus parviflora	N. Amer.	Y	N	N	PK	N	N	N	Good
73. Sophora japonica	Japan, China	Y	N	N	K	PK	N	G	N
74. Syringa persica	Cauc. Reg., Persia	Y	Past	N	N	N	N	N	Fine
75. Syringa vulgaris	Transylv.	Y	Past	N	N	N	N	N	Fine
76. Magnolia obovata purpurea	Gardens	Y	Past	N	PK	N	N	N	Slow
77. Cornus stolonifera	N. Amer.	Y	N	N	N	N	N	N	G
78. Hibiscus syriacus	Old World Calid.	Y	N	N	K	K	N	G	N

TABLE II—Continued

Name of Plant	Habitat	Condition at the Time of Frost			Condition Several Days Later			Adventive Buds	Growth Two Weeks Later
		In Leaf	In Flower	In Bud	Effect on Leaf	Effect on new Shoot	Effect on Bud		
79. Broussonetia papyrifera	Malay, Pacif. Ins.	Y	N	N	K	K	N	G	N
80. Acer laetum rubrum	Orient	Y	?	N	PK	N	N	G	Good
81. Acer saccharinum laciniatum	N. Amer.	Y	?	N	PK	N	N	G	Good
82. Amorpha fruticosa	N. Amer.	Y	N	Y	K	PK	N	N	G
83. Asimina triloba	N. Amer.	Y	P	N	K	K	N	G	Fair
84. Berberis ilicifolia	Magel. Reg.	Y	N	N	PK	PK	N	G	Fair
85. Calycanthus floridus	N. Amer.	Y	N	N	K	N	N	G	Fair
86. Carpinus Betulus	Eu., Cauc. Reg., Persia	Y	N	N	PK	PK	N	G	Fair
87. Cornus Bailey	Gardens	Y	N	N	PK	N	N	G	Good
88. Euonymus atropurpureus	N. Amer.	Y	N	N	PK	N	N	G	Good
89. Gleditschia aquatica	N. Amer.	Y	N	Y	K	PK	N	N	G
90. Gymnocladus canadensis	N. Amer.	Y	N	N	K	PK	N	G	Fair
91. Hydrangea radiata	N. Amer.	Y	N	N	PK	N	N	G	Fair
92. Juglans rupestris	W. N. Amer.	Y	N	N	K	PK	N	G	Fair
93. Leitneria floridana	N. Amer.	Y	N	N	K	N	N	N	Fair
94. Liquidambar styraciflua	N. Amer.	Y	N	N	PK	PK	N	G	Fair
95. Maclura aurantiaca	N. Amer.	Y	N	N	K	N	N	G	Good
96. Magnolia grandiflora	N. Amer.	Y	N	N	K	K	N	G	Fair
97. Magnolia Lennei ×	Gardens	Y	Past	N	PK	N	N	G	Poor
98. Magnolia Umbrella	N. Amer.	Y	N	N	PK	N	N	G	Best
99. Rhus glabra	N. Amer.	Y	N	N	K	PK	N	G	Fair
100. Robinia sp	Hab?	Y	N	N	K	PK	N	G	Fair
101. Sambucus canadensis	N. Amer.	Y	N	N	K	N	N	G	Good
102. Tilia americana	N. Amer.	Y	N	N	PK	N	N	G	Good
103. Ulmus alata	N. Amer.	Y	N	N	PK	N	N	G	Good

later dried and fell to the ground, hung everywhere from the branches. Much of this dried material hung on for weeks, and in maples nearly all summer. This phenomenon of holding on to the dead leaves, or parts of leaves, was extremely interesting. The killing effect of the freeze extended in some cases, as in *Paulownia imperialis,* into the previous year's wood, and nearly always into the new shoots and foliar growth. The variation in its action is very well shown in Table I and II, in which the headings at the top of the columns are taken as questions, and answered for each of the species observed, by *Y* = yes; *N* = no or none; *K* = killed; *PK* = partly killed; *G* = general or good.

From this table we are able to formulate a few general opinions, but mainly the fact that a freeze in its action on vegetation does not always have the same effect on all plants. When we admit this fact then, we are obviously led to the conclusion that there may be all gradations of cause and effect, but that when the cause is the same, as is the case in any definite freeze, then the effect is different for each particular case. In the case of the freeze under consideration it has been possible to group the effects under five rather clearly defined sections.

I. Those species for which complete immunity was recorded.

Clethra alnifolia
Cornus Mas
Cornus stolonifera
Euonymus Sieboldiana
Magnolia Kobus
Magnolia stellata
Quercus imbricaria
Rhus copallina
Syringa persica
Syringa vulgaris

II. Those in which the leaves were only partly frozen.

Acer dasycarpum
Acer laetum rubrum
Acer platanoides
Acer saccharinum laciniatum
Aesculus Hippocastanum
Aesculus parviflora
Cornus Baileyi
Diervilla rosea
Euonymus atropurpureus
Fagus sylvatica purpurea
Halesia tetraptera
Hamamelis virginiana
Hydrangea paniculata
Hydrangea radiata
Ligustrum amurense
Magnolia cordata

Magnolia glauca
Magnolia Lennei ×
Magnolia obovata discolor
Magnolia obovata purpurea
Magnolia Soulangeana
Magnolia Umbrella
Neillia opulifolia
Ostrya virginica
Philadelphus coronarius
Philadelphus grandiflorus
Platanus occidentalis
Pyrus arbutifolia erythrocarpus
Pyrus japonica
Ribes aureum
Taxodium distichum
Tilia americana
Ulmus alata
Ulmus campestris
Viburnum Lentago
Viburnum Opulus sterilis

III. Cases in which the leaf growth was completely killed, and in which there was no apparent injury to the new shoots.

Acanthopanax ricinifolium
Calycanthus floridus
Cercis canadensis
Cladrastis tinctoria
Ginkgo biloba
Hydrangea quercifolia
Koelreuteria paniculata
Leitneria floridana
Liriodendron tulipifera
Maclura aurantiaca
Magnolia acuminata
Magnolia Yulan
Sambucus canadensis
Xanthoceras sorbifolia

IV. Where new leaves were entirely killed, and the new shoots partially killed.

Ailanthus glandulosus
Amorpha fruticosa
Ampelopsis quinquefolia
Aralia spinosa
Berberis ilicifolia
Berberis vulgaris Thunbergii
Carpinus Betulus
Fraxinus americana
Fraxinus Ornus
Fraxinus viridis
Gleditschia aquatica
Gleditschia triacanthos
Gymnocladus canadensis
Juglans rupestris
Lindera Benzoin
Liquidambar styraciflua
Morus alba
Quercus tomentosus
Rhamnus tinctorius
Rhus glabra
Rhus typhina
Robinia sp.
Securinega fluggeoides
Sophora japonica
Spiraea prunifolia
Spiraea Van Houttei

V. New growth of all leaves and shoots completely killed.

Asimina triloba
Broussonetia papyrifera
Catalpa bignonioides
Chionanthus virginicus
Diospyros virginiana
Hibiscus syriacus
Ilex opaca
Magnolia grandiflora
Paulownia imperialis
Pinus austriaca
Rhus cotinus atropurpureus
Rhus toxicodendron

Rosa rugosa
Sassafras officinalis
Taxus baccata
Tsuga canadensis
Vitis vinifera.

SECTION I.

To the first may be added another as a result of this year's (1911) observations, namely *Lonicera involucrata,* which because of its behavior toward a frost of the present year is placed in the same category. The first in the list was the only plant that had not started and so was unaffected and was placed here. While the leaves of the plants recorded in this section were apparently frozen, if we may judge from outward appearances, still they did not show any after effects as were observed to appear in the second section. So that on this evidence alone they have been placed in the list of immune varieties, although it is not taken that they were not frozen. No notes of any peculiarities as to lateness of flowering, or early falling of leaves, etc., were taken later in the season, as will be evidenced in the resultant descriptions of the later sections.

SECTION II.

The enumeration of the species in the second section includes all those plants whose leaves, several weeks after the frost, showed, by the appearance of dried and withered parts, that sections had been killed as a direct or indirect result of the freeze. In nearly all cases the parts of the leaves took on a dried-up, rusty appearance, but in the case of *Cornus Baileyi,* the parts affected were red or black. In *Acer saccharinum laciniatum* the frozen parts dried up and in a few days were broken off by the wind, and only by a close observation could the effect of the freeze be ascertained, but in *Acer platanoides* and *Acer dasycarpum* the dried portions persisted, and during the summer it was possible to distinguish easily the frozen and unfrozen portions of the leaves. By this means it was easily seen from the maples and buckeyes that the major part of the damage occurred on the northwest and north sides of the trees,

partly because the wind was from that direction, and partly, as will be shown later, from another cause. It was observed that the line of demarcation between the frozen and unfrozen portions of the leaf always followed the outline of the latter, so that, minus the frozen portion, the remainder had all the appearances of a true leaf. In the magnolias of this class the frozen margin has acted as a limiting band and the further growth of the leaf has caused an arching of the surface towards the upper side in *Magnolia Lennei* ×, and towards the lower side in *Magnolia acuminata*. The latter, while included in the next section, is perhaps as truly a member of this. It is somewhat later than other members of the Magnoliaceae and had but few leaves exposed at the time of the frost. These were killed. The remainder, whose edges were more or less exposed in the opening bud, showed the effects mentioned above. That there exists a difference in the response of *Acer* and *Magnolia* to the same stimulus must be due to the difference in the maturity of the leaves, the former being probably more mature than the latter, and, consequently, incapable of growth recovery later, while in the latter, growth proceeded even after the leaf had been injured. Some new bud growth was induced, but since the effect was on trees that had already produced their spring crop of leaves, we may conclude that, in the absence of extensive new production of foliage, the productive ability of the remaining leaf surface would be much reduced, and possibly even to such an extent, that its effect would be perceptible in a difference in the size of the annual rings of the trees, or in a reduced activity in the foliar production of the following year, *i. e.*, in earliness and abundance of flowers and leaves, etc.

SECTION III.

We are forced to include this section, though the effect of the frost differs but in degree from the former division, mainly for the reason that we have here a method of

recovery that does not occur in the former case, nor in the one following. We are here concerned only with the origin of new growths, since all exposed growths have been completely killed.

Liriodendron tulipifera. All unfolded leaves were, of course, completely killed. Each leaf is, however, unfolded independently from the former one, and separated from it by the protective opening scales. Those so protected by the scales proceeded to grow, and, although a long time developing completely, were among the first to start, since their growing period was not shortened. The dropping off of foliage later in the season cannot be taken as any frost indication, since this condition occurs nearly every season, probably as a result of smoke injury.

Ginkgo biloba. This is a broad-leaved Gymnosperm which bears its leaves in clusters, of which the outer leaves are the older, and the inner the younger. The new leaves come forth from the center of the cluster one at a time. The freeze killed all of the opened leaves, which then turned brown and hung on until the middle of summer. The new leaves started to come out again within a few days after the frost, but their appearance was largely hidden until after the falling of the killed leaves. At the end of the season their appearance was much better than those of *Liriodendron.*

In both of the above cases the recovery has been rapid solely because the growth of new leaves did not involve the production of new buds, but simply a continuation of development in the buds that had already started, or more correctly, in the development of the uninjured shoot.

Upon the death of many of the leaves on shoots which were not injured, there was an immediate development of the unopened buds, as was the case with *Cercis canadensis* and *Cladrastis tinctoria.* In the latter the leaflets shrivelled up and fell off, the petiole remaining for some weeks, when it, too, fell, or was pushed off by the bud enclosed in its base. In this case we have a development of four separate

series of buds. First, those giving rise to the new shoot; second, those giving rise to the compound leaf; third, those designed for new growth the following year and enclosed within the bases of the leaves; and fourth, the so-called adventitious buds. The same condition also obtains for *Cercis canadensis.* In *Cladrastis* a drying up of the new shoot was noticed near the tip later in the season, but it cannot be said whether this was the result of frost injury, or of the drying effect of an unusually warm summer.

SECTION IV.

This is a somewhat arbitrary division, but by the death of the major portion of the shoot the appearance upon recovery was not as natural as that of the former section, inasmuch as the new leaves had a more clustered appearance. As a matter of fact the freeze cannot be said to have been injurious, any more than we might say the same for a good pruning. By freezing back the ends of the new shoots and entirely killing all new leaf growth, the frost forced new growth from the basal portion of the new shoot, and very largely from the adventitious buds on the old woody portion of the trees and shrubs. The appearance of *Ailanthus glandulosa, Aralia spinosa, Fraxinus, Rhus glabra* and *Rhus typhina,* have never been better than during the season following the freeze. Recovery has been rapid in nearly all cases, and apparently the freeze caused very little harm to this group. *Aralia spinosa,* in a clump on low ground, did not flower at all during the season, while another clump on higher ground flowered profusely. Here again, we cannot definitely place the cause of the difference, for normal growth causes might have resulted in an off year for flowering, as is the case with many of the Rosaceae.

SECTION V.

The last list is perhaps the most interesting of all, since it shows the effect of the freeze upon the new growth of

the Coniferae. The new growths at this time were just started, and they were entirely killed. Of course the old growth of leaves, even if frozen, did not show any effects. In *Broussonetia papyrifera, Catalpa bignonioides, Paulownia imperialis,* the killing was perhaps the most complete of all the trees and shrubs observed, in the case of the latter even extending into the old wood itself. *Magnolia grandiflora* is not hardy in this section, and only thrives when protected in winter. This protection had, of course, been removed and the new growths, as might have been expected, caught the full force of the freeze. It, however, responded well, and growth during the season was nearly normal. *Paulownia,* in the season from April 24 to the time of the first fall frost, October 28, made growths of ten to twelve feet. It, too, is but half hardy and freezes back a little nearly every winter and, strangely, after having passed the winter without freezing back, it did so, in some cases, as a result of the spring freeze, as evidenced by the appearance of the buds on the old wood. This is probably owing to the fact that growing tissue is much more susceptible to cold than is older tissue.

In recording the effects of the freeze on the many trees and shrubs of the list, several reasons have suggested themselves that partially explain why some leaves froze and why some did not. *(a)* Perhaps the most suggestive answer is the one of sap density. It is a well-known law of physics that solutions of different density have different freezing points. The freezing point for water being 32°F., any aqueous solutions, such as plant juices or saps, will have freezing points below that of pure water. We may suppose, then, that differences of sap density may determine the relative resistance to frost or freezing. The answer to this suggestion will occupy the second part of this paper and will not be taken up further here. *(b)* The opening of the buds, and the development of shoots and leaves, extends through a period of several weeks. It is rather interesting to note that those plants unaffected by the frost

are among the first to open and develop their leaves in the spring, as are those only partly affected; and those which have been the hardest hit of all the trees and shrubs, were among the last of all to open and expand their leaves. If it were possible to tabulate the ages of leaves and the order of their opening, we might also get an answer to our question, in that the older the leaf is, the more resistant it becomes. The time consumed in the experiments on freezing point lowering left no opportunity for complete work on this subject, and, except for the general confirmation of this supposition, nothing further has been done on the subject. *(c)* If, then, the ages of the leaves in general become a criterion of their resistance to freezing, then the difference in the ages of the parts of the leaves may account for the partial freezing of maples, etc., noticed in Section II. *(d)* Since the minimum of temperature recorded occurred during the night, we may also assume another cause. In the transformation of starch into sugar, which goes on in the leaf at night (as well as in the daytime), the plant is acquiring for itself a medium with a lower freezing point, for the insoluble starch grains have been changed into sugar, which, in solution, is gradually removed from the leaf. Such being the case the sugar solution would leave the tips of the leaves first and the more concentrated part would be near the center of the leaf. In other words the freezing point is lower at night, or at any time that respiration is greater than photosynthesis, in the center of the leaf than at the edge. It is probable that we have here a possible explanation of the partial freezing noticed in the leaves of *Acer* and *Aesculus*.

2. THE FREEZING POINTS OF LEAF SAPS, DETERMINED ARTIFICIALLY, AND NOTES IN CONNECTION THEREWITH.

At the time when the writer was expecting to make use of the rather cumbersome method of Beckman for determining the freezing point lowering of the cell saps, a paper appeared by Messrs. Dixon and Atkins, (Osmotic Pressure in Plants. Notes from the Botanical School of Trinity Col-

lege, Dublin **2**: 47-83. 1910.) in which they describe the preparation and use of the apparatus used in these experiments. A photograph of the complete apparatus used appears on the accompanying plate. It consists, in the main, of a thermo-couple made up of nickel wire, the ends of which are soldered to copper leads which connect with the terminals of the galvanometer. The junctions are fitted into two small glass tubes into one of which the solution to be frozen is placed (2-3cc.), and in the other, distilled water. The tubes are mounted in such a way that they can be inserted into a convenient vessel, which may be surrounded by the freezing mixture. The couple itself is supported by wooden rods so as to allow of constant stirring during the operation, and for removal and cleaning of both the junctions and the tubes. A reversing key, consisting of an ordinary spring clip, containing the leads from the galvanometer on its inner jaws, and the leads from the couple on the surface of a glass plate, is inserted in the circuit, and the whole submerged in coal oil to eliminate the effect of possible differences of temperature of the terminals of the galvanometer. A diagram of the apparatus is shown in the paper already referred to. The only essential difference in the apparatus as used, occurs in the use of German silver wire in the place of nickel in the thermo-couple, and in the use of a Leeds and Northrup Co. (Philadelphia) galvanometer, instead of the Ayrton-Mather instrument used by Dixon and Atkins. In using the apparatus it is essential that the whole of the couple be at the temperature of ice, for if any part of it remains out of the freezing temperature it acts itself as a couple and results in an inaccurate reading. In practice, after the distilled water has been placed in one of the tubes and the sap to be tested in the other, the holder containing the tubes and the couple is placed in a freezing mixture of salt-water and ice. The mixture surrounding the potato tube of the illustration may be used for this purpose. In a few moments the ice crystals may be seen

forming in the tube containing the distilled water, and shortly after in the tube containing the sap. During this time the liquid in the tubes has been kept stirred by means of the rods holding the thermo-couple. The insulation on the wires of the couple should be examined frequently during the operation so as to remedy any defects caused by the rubbing of the wires on the sides of the tubes in stirring. As soon as crystallization has set in, the holder containing the couple and tubes is put into the potato tube, which all this time has been surrounded by the freezing mixture. The two terminals of the reversing key are connected and the latter inserted in the vessel of coal oil which is kept stirred. When the movements of the mirror of the galvanometer have ceased, the current is reversed, and the reading taken from the other side of the zero. It is well to again reverse and verify the first reading, since if either of the liquids in the tubes should be unfrozen the deflection will increase, or when the liquids are frozen solid a sudden falling off toward the zero will be noticed. The movement of the mirror after the leads have been connected should be steady and then come to a complete stop. Any deviation from this action in the way of erratic movements may be taken as an indication of poor insulation, or over- or under-freezing. A little practice will easily make the operator acquainted with the instrument, and once familiar with it, there will be no difficulty at all in obtaining accurate readings. The deflections of the galvanometer used were not very great, ranging from two to nine millimeters. The tenths of a millimeter were estimated, and, consequently, many of the freezing points given in the tables as identical, may, as a matter of fact, be slightly different. A more sensitive galvanometer is to be desired for accurate and definite results, but for the purpose in view where only the comparison is of importance, the instrument used is quite sufficiently accurate. It has been found that on the jar containing the freezing mixture, ice or frost will usually form. When the

mixture warms the frost first leaves the bottom of the jar, moving in a band toward the top. At the appearance of this symptom it is usually found advisable to refill the jar, as procedure, after this point is reached, is usually slow and uncertain. Again, after connecting the terminals of the reversing key, care should be taken not to handle the stirrer until all traces of oil have been removed from the hands, and splashing of the freezing mixture on the couple should be avoided for very obvious reasons. Perfect cleanliness and care should be exercised throughout the test.

While all of the above is more or less a repetition of the directions for using the apparatus as given by Messrs. Dixon and Atkins, still it seems necessary to repeat such essentials as have been given here.

Altogether, some 110 species of plants (trees and shrubs) have been gathered, and of these only 90 have been finally tested out, owing to the inability of getting sap from some, and to its viscidity in others, thereby preventing its use in the small tubes containing the couple. The plants whose saps, for one reason or another, could not be used in the tests are as follows:—

Crataegus prunifolius	Paulownia imperialis
Pyrus japonica	Sassafras officinalis
Acer platanoides	Ulmus campestris
Aesculus Hippocastanum	Acer laetum rubrum
Quercus imbricaria	Amorpha fruticosa
Pyrus arbutifolius	Euonymus atropurpureus
Acer saccharinum laciniatum	Hydrangea radiata
Ulmus alata	Ampelopsis quinquefolia
Leitneria floridana	Spiraea prunifolia

Tilia americana

Owing to the slow process of sap extraction and the limited time at the disposal of the writer, it was found necessary to carry the experiments along in sections, *i. e.,* the plant leaves were collected from 20 to 30 species at a time, the saps pressed out by means of a vise, after first being bruised in a mortar and wrapped in muslin which had been shrunk several times. Steel plates, which are shown at the left

of the illustration of the instrument used, are a convenient means of conducting the sap from the jaws of the vise to the test tubes. The saps were placed in test tubes, stoppered with cotton, numbered, and then stored in an ice box for approximately twenty-four hours, after which they were frozen in exactly the same order in which they were collected. Since, according to Dixon and Atkins, the saps stored for a period of twenty-four hours have the same freezing points as when freshly extracted, it is assumed that this represents their normal freezing point. But even if this were not so, and the writer has not tried to verify their conclusion, then the results will all be in error in the same direction, and hence are, for our purpose, quite accurate enough. The saps were collected for a period of about a week, fortunately accompanied by rather uniform weather, mostly clear, although cloudy at times, and not accompanied by rains at any time during the experiments. However, the weather was exceedingly dry, and it was at times difficult to obtain perfectly turgid leaves. All of the leaves from which sap was taken, were gathered in full sunlight, and, since they were mostly taken between 2:00 and 4:00 P. M., were from the southwest to the west side of the trees and shrubs. Except for some of the lower growing shrubs, the leaves were gathered at a point from four to five feet from the ground. The variation in color observed by Dixon and Atkins was also clearly shown by the saps after they had been allowed to settle for some time. The color of the sap when first pressed out and that observed after settling, *i. e.*, after the clear liquid had separated out from the chlorophyll and sediment, was quite variable, but since it had, as far as could be determined, exerted no influence on the freezing point lowering or had any relation to the results obtained, the tabulation of these colors has not been observed in this paper.

The times of collection, together with the dates of freezing, are as follows:—

Numbers 1 to 30. Gathered and sap extracted from 11 to 4 P. M., May 13, 1911: saps frozen 1 to 4 P. M., May 15, 1911.

Numbers 31 to 50. Collected May 17, 1911, from 2 to 4 P. M.: frozen 8 to 11 P. M., May 18, 1911.

Numbers 51 to 70. Collected May 18, 1911, from 2 to 4 P. M.: frozen 7 to 11 P. M., May 19, 1911.

Numbers 71 to 92. Collected May 19, 1911, from 2 to 4 P. M.: frozen 9 to 12 A. M., May 21, 1911.

Numbers 93 to 110. Collected May 20, 1911, from 2 to 4 P. M.: frozen 2 to 4 P. M., May 23, 1911.

The saps, with their freezing point lowering in degrees centigrade, are given in the order of their collection. Numbers from 1 to 10 are with thermo-couple number 1, in which one millimeter of deflection equals 0.2361°C., and numbers 11 to 110 are with couple number 2, in which one millimeter of deflection equals 0.29°C., calibrated with the sugar solutions of 10, 20, 30, and 40 grams to 100cc. of water, taking Raoult's lowering for each as respectively 0.58°, 1.16°, 1.74°, and 2.32° centigrade.

		Degrees Centigrade
1.	Cornus Mas	1.771
2.	Hydrangea paniculata grandiflora	1.322
3.	Magnolia Yulan	0.7792
4.	Magnolia cordata	1.2985
5.	Magnolia stellata	1.2513
6.	Magnolia glauca	0.8972
7.	Magnolia acuminata	0.8736
8.	Magnolia Kobus	1.1569
9.	Magnolia Soulangeana	1.2513
10.	Magnolia obovata (M. purpurea)	0.9444
11.	Morus alba	0.87
12.	Vitis vinifera	0.625
13.	Berberis vulgaris Thunbergii	1.45
14.	Crataegus prunifolius (no sap).	
15.	Aralia spinosa	1.363
16.	Ribes aureum	1.16
17.	Hibiscus syriacus	1.102
18.	Viburnum Lentago	1.943

		Degrees Centigrade
19.	Hydrangea quercifolia	0.9325
20.	Cercis canadensis	0.87
21.	Viburnum Opulis sterilis	1.74
22.	Philadelphus gordonianus	1.208
23.	Euonymus Bungeanus	1.305
24.	Pyrus Malus	1.885
25.	Dirca palustris	0.957
26.	Symphoricarpos racemosus	1.624
27.	Euonymus europaeus	1.856
28.	Lindera Benzoin	1.16
29.	Zelkova crenata	1.015
30.	Zanthoceras sorbifolia	1.16
31.	Cornus stolonifera	1.276
32.	Liquidambar styraciflua	1.131
33.	Pyrus japonica (no sap).	
34.	Aesculus austrina (sunlight)	1.073
35.	Aesculus austrina (shade)	0.918
36.	Viburnum dentatum	1.421
37.	Forsythia suspensa	1.073
38.	Halesia tetraptera	1.208
39.	Spiraea bracteata	2.03
40.	Cladrastis tinctoria	1.498
41.	Catalpa bignonioides	1.044
42.	Diervilla rosea var.	0.87
43.	Maclura aurantiaca	1.247
44.	Syringa vulgaris var.	1.74
45.	Acer platanoides (no sap).	
46.	Fagus sylvatica purpurea	1.208
47.	Syringa persica	1.914
48.	Aesculus parviflora	1.131
49.	Aesculus Hippocastanum (no sap).	
50.	Viburnum tomentosum	0.957
51.	Magnolia obovata discolor	1.208
52.	Ginkgo biloba	1.45
53.	Chionanthus virginicus	1.015
54.	Hydrangea paniculata	1.305
55.	Rhus cotinus atropurpureus	1.208
56.	Viburnum Opulis sterilis	1.624
57.	Platanus occidentalis	1.302
58.	Quercus imbricaria (no sap).	
59.	Pyrus arbutifolia (no sap).	
60.	Magnolia Lennei ×	0.741
61.	Euonymus Sieboldianus	1.16
62.	Acer dasycarpum (no sap).	

		Degrees Centigrade
63.	Rhamnus tinctoria	1.74
64.	Ilex opaca	0.596
65.	Hamamelis virginica	1.16
66.	Clethra alnifolia	1.073
67.	Philadelphus coronaria	1.131
68.	Philadelphus grandifolius	1.16
69.	Koelreuteria paniculata	1.131
70.	Sambucus canadensis	1.566
71.	Liriodendron tulipifera	1.305
72.	Acer saccharinum laciniatum (no sap).	
73.	Ulmus alata (no sap).	
74.	Carpinus Betula	1.479
75.	Magnolia Umbrella	0.87
76.	Leitneria floridana (no sap).	
77.	Ostrya virginica	1.45
78.	Diospyros virginiana	1.015
79.	Taxodium distichum	1.073
80.	Asimina triloba	0.625
81.	Tilia americana (no sap).	
82.	Fraxinus Ornus	1.363
83.	Ligustrum amurense	1.74
84.	Broussonetia papyrifera	1.16
85.	Fraxinus americana	0.712
86.	Ailanthus glandulosus	1.16
87.	Paulownia imperialis (no sap).	
88.	Rhus typhina	1.015
89.	Rhus glabra	1.276
90.	Sassafras officinalis (no sap).	
91.	Ulmus campestris (no sap).	
92.	Acer laetum rubrum (no sap).	
93.	Gymnocladus canadensis	1.74
94.	Fraxinus viridis	1.208
95.	Cornus Baileyi	1.276
96.	Neillia opulifolia	1.015
97.	Gleditschia aquatica	1.305
98.	Amorpha fruticosa (no sap).	
99.	Euonymus atropurpurea (no sap).	
100.	Berberis ilicifolia	1.566
101.	Calycanthus floridus	1.392
102.	Rhus copallina	1.305
103.	Juglans rupestris	1.015
104.	Magnolia grandiflora	1.044
105.	Hydrangea radiata (no sap).	
106.	Securinega fluggeoides	1.566

		Degrees Centigrade
107.	Ampelopsis quinquefolia (no sap).	
108.	Spiraea prunifolius (no sap).	
109.	Gleditschia triacanthos	1.595
110.	Spiraea Van Houttei	1.566

The following table is the arrangement of the species in the order of their freezing point lowering. The numbers following the names of the species represent the respective section to which they were referred in the tentative lists arranged directly after the freeze of 1910:—

		Degrees Centigrade
1.	Spiraea bracteata	2.03
2.	Viburnum Lentago (2)	1.943
3.	Syringa persica (1)	1.014
4.	Pyrus Malus	1.885
5.	Euonymus europaeus	1.856
6.	Cornus Mas (1)	1.7708
7.	Gymnocladus canadensis (4)	1.74
8.	Ligustrum amurense (2)	
9.	Rhamnus tinctoria (4)	
10.	Syringa vulgaris var. (1)	
11.	Viburnum Opulis sterilis (2)	
12.	Viburnum Opulis sterilis (2)	1.624
13.	Symphoricarpos racemosus	
14.	Gleditschia triacanthos (4)	1.595
15.	Spiraea Van Houttei (4)	1.566
16.	Securinega fluggeoides (4)	
17.	Berberis ilicifolia (4)	
18.	Sambucus canadensis (3)	
19.	Cladrastis tinctoria (3)	1.498
20.	Carpinus Betulus (4)	1.479
21.	Ostrya virginica (2)	1.45
22.	Ginkgo biloba (3)	
23.	Berberis vulgaris Thunbergii (4)	
24.	Viburnum dentatum	1.421
25.	Calycanthus floridus (3)	1.392
26.	Platanus occidentalis (2)	
27.	Fraxinus Ornus (4)	1.363
28.	Aralia spinosa (4)	
29.	Hydrangea paniculata grandiflora (2)	1.3222
30.	Rhus copallina (1)	1.305
31.	Gleditschia aquatica (4)	
32.	Liriodendron tulipifera (3)	

		Degrees Centigrade
33.	Hydrangea paniculata (2)	
34.	Euonymus Bungeanus	
35.	Magnolia cordata (2)	1.2985
36.	Cornus Baileyi (2)	1.276
37.	Rhus glabra (4)	
38.	Cornus stolonifera (1)	
39.	Magnolia Soulangeana (2)	1.2513
40.	Magnolia stellata (1)	
41.	Maclura aurantiaca (3)	1.247
42.	Fraxinus viridis (4)	1.208
43.	Rhus cotinus atropurpureus (5)	
44.	Magnolia obovata discolor (2)	
45.	Fagus sylvatica purpurea (2)	
46.	Halesia tetraptera (2)	
47.	Philadelphus gordonianus	
48.	Ailanthus glandulosus (4)	1.16
49.	Broussonetia papyrifera (5)	
50.	Philadelphus grandiflorus (2)	
51.	Hamamelis virginica (2)	
52.	Euonymus Sieboldianus (1)	
53.	Zanthoceras sorbifolia (3)	
54.	Lindera Benzoin (4)	
55.	Ribes aureum (2)	
56.	Magnolia Kobus (1)	1.1569
57.	Koelreuteria paniculata (3)	1.131
58.	Philadelphus coronarius (2)	
59.	Aesculus parviflora (2)	
60.	Liquidambar styraciflua (4)	
61.	Hibiscus syriacus (5)	1.102
62.	Taxodium distichum (2)	1.073
63.	Clethra alnifolia (1)	
64.	Forsythia suspensa	
65.	Aesculus austrina (sunlight)	
66.	Magnolia grandiflora (5)	1.044
67.	Catalpa bignonioides (5)	
68.	Juglans rupestris (4)	1.015
69.	Neillia opulifolia (2)	
70.	Rhus typhina (4)	
71.	Diospyros virginiana (5)	
72.	Chionanthus virginicus (5)	
73.	Zelkova crenata	
74.	Viburnum tomentosum	0.957
75.	Dirca palustris	
76.	Magnolia obovata (M. purpurea) (2)	0.9444

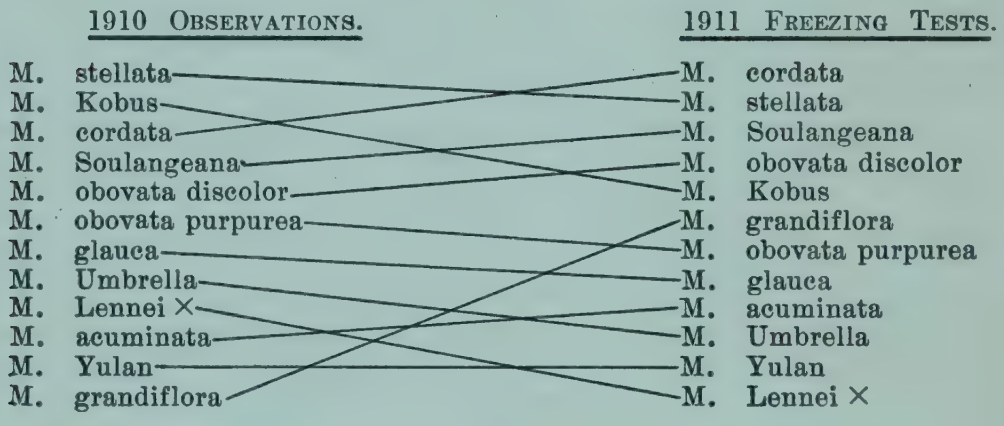
		Degrees Centigrade
77.	Hydrangea quercifolia (3)	0.9325
78.	Aesculus austrina (shade)	0.918
79.	Magnolia glauca (2)	0.8972
80.	Magnolia acuminata (3)	0.8736
81.	Magnolia Umbrella (2)	0.87
82.	Diervilla rosea var. (2)	
83.	Cercis canadensis (3)	
84.	Morus alba (4)	
85.	Magnolia Yulan (3)	0.7792
86.	Magnolia Lennei × (2)	0.741
87.	Fraxinus americanus (4)	0.712
88.	Asimina triloba (5)	0.625
89.	Vitis vinifera (5)	
90.	Ilex opaca (5)	0.596

By dividing the above list, in which the species are arranged in descending order, according to the respective freezing point lowerings, into halves, and by listing under each half the number of species therein belonging to the sections outlined in the early part of this paper, the following arrangement results:—

First Half.	Second Half.
6 of Section I.	3 of Section I.
13 of Section II.	13 of Section II.
6 of Section III.	6 of Section III.
13 of Section IV.	7 of Section IV.
0 of Section V.	10 of Section V.

In the above the absence of any of Section V from the first half is very noticeable, and only a small fraction of a degree keeps the three of Section I in the second half. Those belonging to Section III, as might be expected, are evenly distributed in both halves, since they represent the intermediate position between Sections I and V. However, the large number of Section II in the second half, and the large number of Section IV in the first half seems to be rather inconsistent, although when we take into consideration the fact that these sections represent purely arbitrary divisions, it is not to be given much thought.

The greatest freezing point lowering, 2.03°C., and the least freezing point lowering, 0.596°C., constitute but a small range in temperature. Nevertheless, within this range there occur several similarities of conduct under varying conditions, to which it may be well to call attention here. *Viburnum Opulus sterilis,* collected and frozen on separate days from different plants, vary but 0.116°C. from each other. However, in *Aesculus austrina,* collected in the sun, and that collected in the shade at the same time and from the same tree, there is a difference of 0.155°C., the lowest freezing point lowering being for the sample from sunlight. The maximum lowering of 1.2985°C., and the minimum of 0.741°C., for *Magnolia cordata* and *Magnolia Lennei* × respectively, indicates a wide difference in the behavior of this group. Their relations from the foregoing table are here submitted for more graphic comparison:—



Taking the observations of 1911 as a basis, there would seem to be a very close relation between the behavior of this group in actual and artificial conditions. With the exception of *Magnolia grandiflora* and, perhaps, of *Magnolia Kobus,* the lines connecting the members of the two groups diverge but little. The impression seems to be given that within species of the same genus, differences in the density of the cell sap result in differences in the resistance of the plants to freezing weather, and the relative relation of the species in this respect may be determined by a knowledge of the freezing points of their cell saps, obtained under

the same conditions of light, exposure, etc. It is quite probable that the differences noted above may have been caused by the failure, in either of the two seasons, to take account of the relative protective positions of the trees from which specimens for sap determination were taken.

3. CONCLUSIONS.

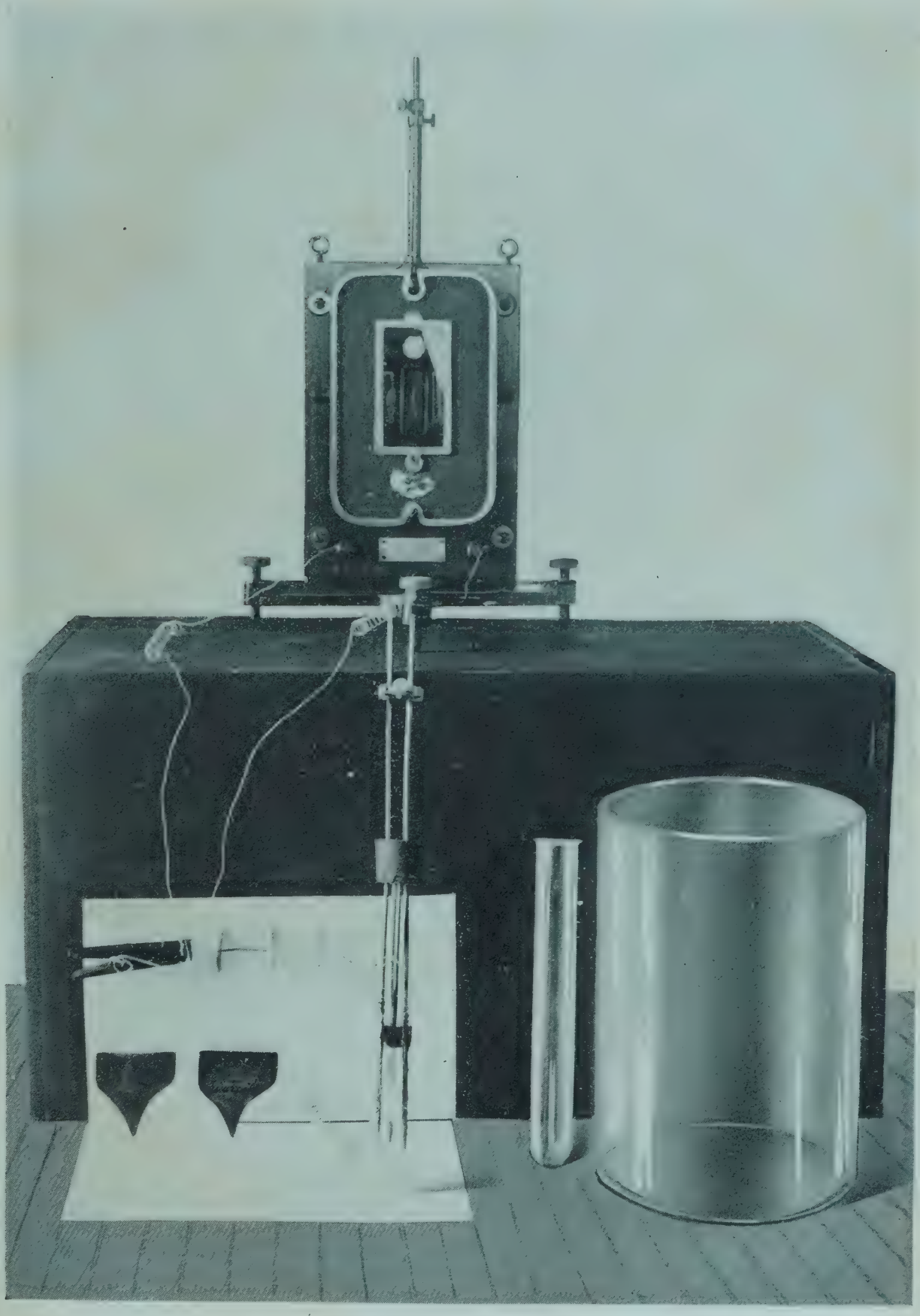
Our conclusions are based upon two series of investigations: one, the observations of the effect of a freeze on many trees and shrubs; the other, the artificial freezing of the leaf saps of the above trees and shrubs. The first is more or less arbitrary, being based mostly on external appearances; the second is hypothetically accurate. From a comparison of these two sets of data we are led to the following conclusions:—

1. That extreme differences in sap density, in general, are accompanied by a corresponding difference in their resistance to freezing.

2. That exceptions to this general rule are probably due to differences of cell structure, and other causes that may enter in, as protective location, etc.

3. That where cell structure is the same, the densities of the cell saps indicate their relative hardiness, as in the magnolias.

4. That in plants of the same genus, or varieties of the same species, differences in sap density correspond to differences in their resistance to freezing.



FREEZING APPARATUS.

THE ARM OF THE GALVANOMETER HAS BEEN REMOVED.

A MONOGRAPH OF THE NORTH AMERICAN *USNEACEÆ*.

BY R. HEBER HOWE, JR.

PART I.

It is my purpose to issue in parts a monograph of the North American *Usneaceæ*. The work will embody the results of my studies of the family which have extended over a period of nearly ten years. These studies have been made afield in all the distributional zones of our area, and with the aid of many important herbaria in both this country and Europe. It is hoped this work will supply a manual for future research in this most difficult[1] and interesting family of plants.

CLASSIFICATION.

In my paper on the Classification de la Famille des *Usneaceæ* dans l'Amerique du Nord (Thèses présentées a la Faculté des Sciences de l'Université de Paris pour obtenir le titre de Docteur de l'Université, Série 60, ordre 71, 1-32: 1912; and Mycologia, **4**: 152-153. 1912) will be found a complete study of the taxonomy of this family, which I have followed here with only a few minor changes. Below is given the working hypothesis on which this classification is based.

Division:

THALLOPHYTES.

Origin.

(Symbiotic)

Subdivision: (*a*) Composition of the thallus.

Class: (*a*) Method of spore production.

[1] Harmand, Lich., France, 372. 1907.

Subclass: (*a*) Condition of the mycelium. (*b*) Distribution of the gonidia.

Order: (*a*) Type of apothecia.

Suborder: (*a*) General form of apothecia. (*b*) Development of spores.

Group: (*a*) General form of the thallus. (*b*) Habit of growth—attachment.

Family: (*a*) Form of thallus. (*b*) Structure of apothecia. (*c*) Type of gonidia.

Tribe: (*a*) Type of cortical structure. (*b*) Type of medulla.

Genus: (*a*) Structure of the thallus. (*b*) Type of spore. (*c*) Form of apothecial margin.

Section: (*a*) Number. (*b*) Form. (*c*) Color of the spores.

Series: (*a*) Peculiarities of thalline structure.

Species: (*a*) Differentiation of the thallus or spores.

Variety: (*a*) Constant morphological variation of the thallus or spores.

Phase: (Without name) (*a*) Type of reproduction. (*b*) Color—dicroic. (*c*) Contingent conditions.

The genera that here constitute the Family *Usneaceæ* have been assembled under groups variously titled and of widely varying generic composition. First included (plus p.) in Linneus' *Filamentosi,* they have appeared since under *Ramalodei, Thamnoblasteæ, Parmeliaceæ, Radiatæ,* etc. The grouping of the present genera has caused, except in the case of *Teloschistes,* but slight taxonomic dislocation. The main question for argument seems to lie in whether the grouping of genera had best follow phylogenetic indications supplied by spore characters[2] or by thalline affinities. *Teloschistes flavicans* var. *capensis* (L. fil) Nyl. certainly argues a close thalline relation in this recently introduced genus, and it is interesting to note that Hoffmann called this a species under the name *Usnea capensis.* (See diagram.)

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[2] See Hue, Bull. Soc. Bot. France **58** : 1911.

GYMNOCARPALES

Simple Spores	Bilocular Spores	Polari-locular Spores	Pluri-locular Spores	Muriform Spores		
Usnea, Letharia, Dufourea, Coelocaulon, Alectoria, Cetraria, Thamnolia?, Siphula? (lecanorine)	Ramalina lecanorine	Teloschistes lecanorine	Roccella, Schizopelte, Combea, Dendrographa (lecidiene)	Oropogon lecanorine	Radiatæ	(Filamentose) / Fruticose
Cladonia, Philophorus lecidiene			Stereocaulon lecidiene		Stratosi Radiatæ	
	Anaptychia, Pseudophyscia lecanorine				Substratosæ	
Platysma, Evernia, Parmelia (lecanorine), Gyrophora lecidiene, etc.	Parmelia, Pyxine, Physcia (lecanorine), etc.	Xanthoria lecanorine etc.	Peltigera, Sticta (lecanorine), etc.	Umbilicaria lecanorine etc.	Foliose	
		Placodium			Stratosæ	(Squamose)
Lecanora lecanorine, Lecidea lecidiene, etc.	Rinodina lecanorine, Buellia lecidiene, etc.	Placodium lecanorine etc.	Bilimbia lecidiene etc.	Rhizocarpon lecidiene etc.		Crustose

Phylogenetic development

Thalline development

It has been recently well said that "reproduction by spores plainly decreased greatly when the symbiotic relationship was taken on, and the result has in all probability been a considerable physiological degeneration of the spores in the course of phylogenetic development." The lichens, unlike the fungi, have other methods of reproduction, and we must not lay too much stress on the structures of the largely abortive fruit when after all it is the thallus of a lichen that is unique,—and we are considering the classification of them and not of true fungi.

In the present classification the thalline method has been followed. This should not blind one to the fact that the radial *Teloschistes* have undoubtedly their closest phylogenetic relatives (as indicated by the spore) in the polaribilocular *Xanthorias, Placodiums,* etc. Throughout this classification the fruit characters are given the first *(Gymnocarpales)* and large importance,—but as in the *Phanerogams* their *protophytic* characters are not employed. Phylogenetically, probably, *Usnea* and *Ramalina, Alectoria* and *Oropogon,* are distinct, yet it seems hardly wise to separate these radial and lecanorine genera. The inclusion of *Teloschistes* by M. Hue has been an innovation, rather than illogical. To introduce into the family, as the thalline extremists have done, the lecidiene genus *Roccella* would be a vastly greater step;—contradictory to the apothecial, as their inclusion with the *Graphidineæ* has been to thalline affiliation. Medio tutissimus ibis.

CHEMICAL CONSIDERATIONS.

This family has been shown, particularly by the late Dr. W. Zopf,[3] to produce several lichen acids. These have been used as aids in classification, though for them can be claimed comparatively little practical value. The following varieties have been attributed to the *Usneaceæ*:

[3] Die Flechtenstoffe, 1907.

Usnea	*Letharia*	*Ramalina*	*Thamnolia*	*Coelocaulon*
Usnaric acid Barbatinic acid Plicatic acid Hirtinic acid Hirtellinic acid Pikrusnidic acid Usnellinic acid Dextrousrinic acid	Usniric acid Vulpinic acid Atranoric acid Divaricatic acid	Usniric acid Ramalic acid Ramalinic acid Evernic acid Scopulerie acid Salazinic acid Atranoric acid	Thamnolic acid	Protolichesterin acid

Cetraria	*Alectoria*	*Teloschistes*
Usniric acid Protolichesterin acid Fumar-Protocetrarie acid Paralichesterin acid	Barbatinic acid Atranoric acid Laevonsuinic acid Alectoric acid Bryopogoric acid Psoromic acid	Parietin acid

The question of the value of chemical tests has long been one of contention, though, undoubtedly, they may often be helpful. When, as in the case of *Ramalina sub-*

farinacea, they are unaccompanied by marked morphological or structural differences, it seems decidedly unwise to employ them alone for the establishment of species. If the tests be made under the microscope, it will be seen at once that only those with iodine affect the actual lichen tissue—the others affecting only excreted or extraneous matter. Though the former can be considered perhaps a part of the lichen, as much as the waxy excretion of the phanerogamic glaucous leaf, yet it is hardly an important structural indication of differentiation. Again, it is well-known that certain lichen acids which are the cause of these color reactions (potash with atranoric acid, etc.), are developed occasionally only in young thalli growing on certain substrata (i. e., *Xanthorina parietina* on sandstone), and such "considerations should make one very wary in placing reliance on these color reactions for the purposes of classification."

SYNONYMY.

As the complete and accurate synonymy of a large number of lichens is impossible to compile (l. c. 6), a comparison of all types being necessary, I am giving here only the "name finding" synonymy and any other titles which have been used to designate the species in North American works. These I have included only when absolutely sure of their synonymous character.

NOMENCLATURE.

The International Botanical Code has been followed. I have indicated, however, the type species of each genus. No forms, states or phases are recognized by title. Much lichenological nomenclature can hardly be termed binomial.

TYPE MATERIAL.

One of the most important portions of my work on this family has been the investigation of types, photographs of which accompany the species here described. In my former

papers on the genera *Usnea*,[4] *Evernia*,[5] *Alectoria*[6] and *Oropogon*,[7] will be found a complete history of the type specimens, augmented by a few special papers (Bull. Torr. Bot. Club **37**: 605-609. 1910 and l. c.), including a report on the Linnean Herbarium (Bull. Torr. Bot. Club **38**: 199-203. 1912). It has been only through the great kindness and generosity of the curators of the herbaria of both this country and Europe that this knowledge, which I consider vital, has been gained. Below is given the list of authors, museums and their curators, to whom I am gratefully indebted.

ACHARIUS: Universitetets Botaniska Institution, Helsingfors, Finland—Dr. Fred. Elfving. Linnean Society of London, Burlington House, London, England—Dr. B. Daydon Jackson.

ANZI: British Museum of Natural History, London, England—Dr. A. B. Rendle. Museum d'histoire naturelle, Paris, France—Dr. L. A. Mangin.

BELLARDI: Istituto ed Orto Botanico della R. Universita, Turin, Italy—Dr. O. Mattirolo.

COSSON: Museum d'histoire naturelle, Paris, France—Dr. E. Perrier.

CROMBIE: British Museum (Natural History), London, England—Dr. A. B. Rendle.

DE NOTARIS: Istituto e orto Botanico, Rome, Italy—Dr. R. Pirotta.

DILLENIUS: Botanic Gardens, Oxford, England—Dr. S. H. Vines.

DUBY: Universitets Botaniska Institution, Upsala, Sweden—Dr. H. O. Juel.

EHRHART: Royal Botanic Gardens, Kew, London, England—Col. D. Prain. Linnean Society of London, Burlington House, London, England—Dr. B. D. Jackson.

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[4] A Manual of the Genus *Usnea*, as represented in North and Middle America, north of the 15th parallel. Bull. Torr. Bot. Club **37**: 1–18. 1910.

[5] The Genus *Evernia* as represented in North and Middle America. Bot. Gazette **51**: 431–442. 1911.

[6] American Species of *Alectoria* occurring north of the 15th parallel. Mycologia **3**: 106–150. 1911.

[7] Mycologia **4**: 152–156. 1912.

FÉE: Botanisches Institut, Strassburg, Germany—Dr. H. Kniep. Museu Nacional do Rio de Janeiro, Brazil, So. Amer.—Dr. Neves Armond.

FRIES, E.: Universitets Botaniska Institution, Upsala, Sweden—Dr. H. O. Juel.

FRIES, TH.: Universitets Botaniska Institution, Upsala, Sweden—Dr. H. O. Juel.

HERRE: Leland Stanford Jr. University—Mr. J. McMurphy.

HOOKER: Royal Botanic Gardens, Kew, London, England—Col. D. Prain.

LENORMAND: Institut Botanique, Caen, France—Dr. O. Lignier.

LINNEUS: Linnean Society of London, Burlington House, London, England—Dr. B. D. Jackson.

MULLER—Arg.: Herbier Boissier, Chambésy, Switzerland—Dr. G. Beauverd.

NOEHDEN: British Museum (Natural History), London, England—Dr. A. B. Rendle.

NORBLIN: Museum d'histoire naturelle, Paris, France—Dr. L. A. Mangin.

NYLANDER: Universitetets Botaniska Institution, Helsingfors, Finland—Dr. Fred. Elfving. Museum d'histoire naturelle, Paris, France—Dr. Mangin.

PERSOON: 'S Rijks Herbarium, Leiden, Holland—Dr. W. J. Longmans.

RETZIUS: Botaniska Institutionen, Lund, Sweden—Dr. O. R. Holmberg.

SCHREBER: Kgl. pflanzenphysiologisches Institut, Munich, Germany—Dr. O. Renner.

STIZENBERG: U. S. National Museum, Washington, D. C., U. S. A.—Drs. J. N. Rose and W. R. Maxon.

SWARTZ: Riksmuseets Botaniska Afdelning, Stockholm, Sweden—Dr. C. A. M. Lindman.

TAYLOR: Boston Society of Natural History, Boston, Mass., U. S. A.—Mr. C. W. Johnson. Royal Botanic Gardens, Kew, London, England—Col. D. Prain.

TUCKERMAN: Botanic Museum, Cambridge, Mass., U. S. A.—Dr. W. G. Farlow. Sprague Herbarium, Boston Society of Natural History, Boston, Mass., U. S. A.—Mr. C. W. Johnson.

VILLARS: Museum Ville de Grenoble, Grenoble, France—Dr. M. Mirande.

ORIGINAL DESCRIPTIONS.

The original descriptions are always given, and have in all cases been copied from the original work. It seems desirable, considering their varied sources, to bring them here under one cover.

FIGURES.

The references refer to the plates which accompanied the original descriptions. If no plate was published with the original description to the earliest figure following the publication of the name. Of the Linnean species the Dillenian plate is always cited in brackets, as they were invariably referred to by Linneus, and represent his conception.

The present work is illustrated with half-tones of the type specimens, if found still preserved in herbaria. All the plates heretofore published in connection with my former articles are again reproduced with the kind permission of the editors of the Bulletin of the Torrey Botanical Club, Botanical Gazette, Mycologia, Prof. Bruce Fink, and M. Bonnier of the Sorbonne, Paris.

KEYS.

The keys, it is hoped, will be valuable, augmented as they are by the classification, and the diagnoses for each species and variety. Even the best and most logical keys are to the author's mind, however, never as satisfactory as brief italicized diagnoses, which have been based as far as possible on external features.

DESCRIPTIONS.

The descriptions have been compiled to allow comparison. The plants of a genus are always treated under definite captions, *i. e.,* thallus, cortex, lacinia or primary and secondary branches, fibrils, apothecia, spores, etc.

DISTRIBUTION.

As I have already stated the distribution in North America of the species of this family follow to a marked degree the

Life Zones of which a map is given. This is due, no doubt, to the fact that they are not (or little) dependent on soil conditions. In giving the distribution I have, however, not only stated political and altitudinal limits as far as possible, but a diagram accompanies each species.

SUBSTRATA.

Though in my own herbarium labels I have indicated whenever possible the exact character of the substratum, and this method has also been followed in many of the modern herbaria examined, yet it does not seem either practical or particularly valuable to list minutely the substrata for lichen species. I have therefore employed the following general terms: coniferous or deciduous trees; dead timber—fallen trees, etc.; old wood—fence rails, roofs, etc.; stones, boulders and ground, including actual soil. The question of exposure is also important. My observations go to prove that strongly lighted or sunny exposures generally produce the fruited (apothecial) conditions, whereas sorediate or soraliate phases are found most commonly in shaded or humid places. Yellow lichens are almost invariably sunlight species, which their color may explain (l. c. 26). Rigid types are frequently maritime.

HERBARIA.

Below is given a list of Herbaria I have examined with the names of the curators, to whom I am gratefully indebted. It has been absolutely necessary in such a variable family to examine an enormous amount of material. It is only by so doing that one can judge the true value of species and varieties. In two of my former papers on the genera, I gave lists of all material examined; such a procedure, though of distinct value is, nevertheless, impractical in the present extended work.

U. S. National Museum, Washington, D. C.—Drs. J. N. Rose and W. R. Maxon.

Academy Natural Sciences, Philadelphia, Pa.—Dr. S. Brown.

New York Botanical Gardens, New York, N. Y.—Dr. N. L. Britton.

Wellesley College, Wellesley, Mass.—Dr. L. W. Riddle.

Prof. Bruce Fink's, Miami University, Oxford, Ohio.

Portland Society of Natural History, Portland, Me.—Mr. A. H. Norton.

Sprague, Boston Society of Natural History, Boston, Mass.—Mr. C. W. Johnson.

Cummings, Wellesley College, Wellesley, Mass.—Dr. L. W. Riddle.

Boston Society of Natural History, Boston, Mass.—Mr. C. W. Johnson.

Dr. L. W. Riddle's, Wellesley, Mass.

Sullivant Moss Chapter, Washington, D. C.—Miss M. F. Miller.

University of Maine, Orono, Me.—Dr. Mintin A. Chrysler.

Williams College, Williamstown, Mass.—Dr. S. F. Clarke.

Sheffield Scientific School, New Haven, Conn.—Dr. A. W. Evans.

Johns Hopkins University, Baltimore, Md.—Dr. D. S. Johnson.

Taylor, Boston Society of Natural History, Boston, Mass.—Mr. C. W. Johnson.

Dr. H. E. Hasse's, Sawtelle, California.

Mr. C. C. Plitt's, Baltimore, Md.

Carnegie Museum, Pittsburg, Pa.—Dr. O. E. Jennings.

Brown University, Providence, R. I.—Dr. J. F. Collins.

Canadian Geological Survey, Ottawa, Canada—Prof. John Macoun.

Dr. A. C. Herre's, Oakland, California.

Cosson, Museum d'histoire naturelle, Paris, France.—Dr. E. Perrier.

British Museum (Natural History), London, England—Dr. A. B. Rendle.

Museum d'histoire naturelle, Paris, France—Dr. L. A. Mangin.

Montagne, Museum d'histoire naturelle, Paris, France—Dr. L. A. Mangin.

Royal Botanic Gardens, Kew, London, England—Col. D. Prain.

Linnean, Burlington House, London, England—Dr. B. Daydon Jackson.

Dillenian, Botanic Gardens, Oxford, England—Dr. S. H. Vines.

Acharian (in p.), Burlington House, London, England—Dr. B. Daydon Jackson.

Smith, Burlington House, London, England—Dr. B. Daydon Jackson.

EXSICCATI.

Under each species I am citing all examples published in North American exsiccati. Below is given the list of exsiccati examined of both North America and European distribution:

Canadian Lichens, prepared by John Macoun, 18—

Lichenes Boreali-Americani, Cummings, Williams, Seymour, 1894-1899.
Decades North American Lichens, Cummings, 1899-1903.
Lichenes Exsiccati, Mr. G. K. Merrill, 1909—
Lichenes Nova Angliæ, Howe, 1909-10.
Kryptogamæ Exsiccati, Dr. A. Zahlbruckner, 1894—
Lichenes Rariores Exsiccati, Dr. A. Zahlbruckner, 1902-04.
Lichenes Rariores Veneti, Anzi, 1863.
Lichenes Etruriæ Rariores Exsiccati, Anzi, 1863.
Lichenes Sueciæ Exsiccati, E. Fries, 1818-1827.
Lichenes Americæ septentrionalis Exsiccati, Tuckerman, 1847-1854.
Mexican Lichens, Pringle, 18—
Lichens of Eastern North America, Small, 189—
Lichenes arctici collecti æstate, Fellman, 1863.
Herbarium Lichenum Fenniæ, Norrlin et Nylander, 1875-1882.
Lichenes Scandinaviæ Exsiccati, Th. Fries, 1859-1865.
Die Flechten Europas, Hepp, 1853-1867.
Lichenes Helvetici Exsiccati, Schaerer, 1823-1852.
Lichenes Europæi Exsiccati, Rabenhorst, 1855-1879.
Lichenes Sueciæ Exsiccati, Stenhammar, 1860-1865.
Lichens de la Normandie, Malbranche, 1863-1869.
Lichenes Floræ Rossiæ, Elenkin, 1906-1907.
Lichenes Exsiccati circa Cantabrigiam, Larbalestier, 1896.

DIVISION: *Thallophytes.*

SUBDIVISION: *Lichenes* (Tournefort) Micheli.

CLASS: *Ascolichenes* A. Zahlb.

Lichens in which the spores are enclosed in asci.

SUBCLASS: *Lichenaceæ* Nyl.

Thallus nongelatinous, gonidia heteromerous.

ORDER: *Gymnocarpales* (Luyken) Fr.

Apothecia open, epithecium visible.

SUBORDER: *Cyclocarpineæ* Wain.

Apothecia discoid; spores maturing in the asci: no capillitium.

GROUP: *Radiatæ* Hue.

Thalline structure radially symmetrical; thallus attached to the substratum without rhizoids.

FAMILY: *Usneaceæ* Eschw. System. Lich. 23. 1824.

DIAGNOSIS: Thalline cortex contiguous: cylindrical or angular, bifacial or deformed.
Apothecia lecanorine. Gonidia *Protococcoid.*

DESCRIPTION: Thallus fruticose or filamentous, radial or bifacial; gray, yellow, green, brown or black. Gonidia *Protococcoid.* Apothecia lecanorine (having a thalloid exciple), marginate or emarginate, entire, crenulate or ciliate, disk concolorous, yellow, brown, or black. Spores monoblast, bilocular to muriform—hyaline or colorate.

The *Usneaceæ,* from their size and conspicuous character, are commonly recognized, though often by the laity as mosses. They are represented in all the zones of our area, and are found growing on earth, rocks and trees. Like most lichens, for their highest development they need not only moisture, but abundant light. Economically they possess little practical value, though certain species have been used for food *(Cetraria islandica),* for medicine *(Usnea florida)* and for dyes *(Letharia vulpina).* It is doubtful if they are appreciably injurious to trees. They are evidently very sensitive to atmospheric conditions, and their presence is indicative of pure air, for the partial cleansing of which they are no doubt responsible. As only a few species *(Ramalina, Usnea)* of this family are rock forms, they play little part in the disintegrations which produce soil.[9]

TRIBE: *Usneeæ* Hue Lich. extra Europ. 31. 1899.

Cortical structure complex (decomposed). l. c. 11.28.
Medulla arachnoid.

(To be continued.)

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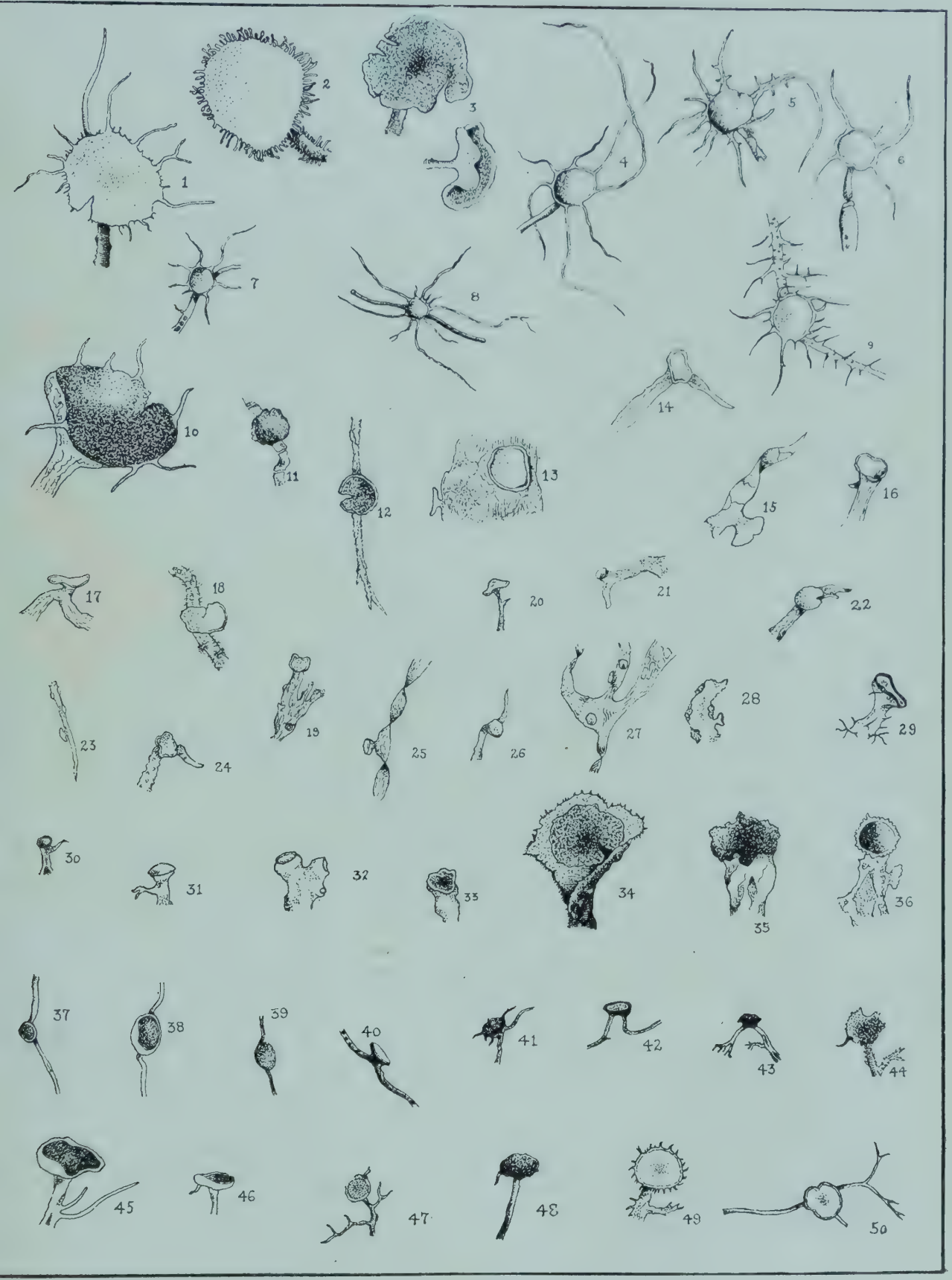
[9] See Herre, Proc. Wash. Acad. **12** : 218. 1910—*Ramalina homalea.*

EXPLANATION OF PLATE.

1. Usnea florida.
2. " florida var. strigosa.
3. " sulphurea.
4. " longissima.
5. " angulata.
6. " barbata.
7. " cavernosa.
8. " trichodea.
9. " plicata.
10. Letharia vulpina.
11. " divaricata.
12. " thamnodes.
13. Ramalina fraxinea.
14. " fastigiata.
15. " homalea.
16. " populina.
17. " calicaris.
18. " ceruchis.
19. " polymorpha.
20. " rigida.
21. " Duriaei.
22. " stenospora.
23. " farinacea.
24. " complanata.
25. " usneoides.
26. Ramalina Montagnei.
27. " reticulata.
28. " pollinaria.
29. " crinita.
30. " dilacerata.
31. " geniculata.
32. " pusilla.
33. Dufourea arctica (after Reinke).
34. Cetraria islandica.
35. " cucullata.
36. " nivalis.
37. Alectoria sarmentosa var. luteola.
38. " sarmentosa.
39. " jubata.
40. " Fremontii.
41. " oregana.
42. " loxensis.
43. Coelocaulon californicum.
44. " aculeata.
45. Alectoria ochroleuca.
46. " lata.
47. " pubescens.
48. " tristis.
49. Teloschistes chrysophthalmus.
50. " flavicans.



APOTHECIA OF THE FAMILY USNEACEAE (slightly reduced).

THE EFFECT OF TOLUOL AND CS_2 UPON THE MICRO-FLORA AND FAUNA OF THE SOIL.[1]

BY P. L. GAINEY.

The work herein presented was undertaken to determine whether the theory advanced by Russell and Hutchinson (1) was adequate to explain the phenomena subsequent to "partial sterilization" when applied to local soils. As is well-known, this theory rests upon the destruction of protozoa that inhibit the maximum development of soil bacteria and hence prevent the rendering of soil nitrogen available. Certain results obtained have been of such a nature that the writer feels justified in bringing them before the public. This is done with the hope that investigations along the same lines by others may either controvert or substantiate the facts herein presented. These investigations are by no means complete, and hence this paper is more of the nature of a preliminary report.

The recent appearance of Fred's (2) article, in which an excellent review of most of the literature bearing upon the subject was given, renders a complete historical résumé unnecessary, hence only a summary of the more important contributions will be given. Readers are referred to the article just mentioned for a more complete review.

The evidence brought forth thus far as to the effects of "partial sterilization" upon the microörganisms of the soil has, with one or two exceptions, been very fragmentary. Most investigators have concerned themselves with the effects of such treatment upon crop production. However, the writer has been able to glean from the literature the following expressions regarding such treatment upon the more important soil phenomena.

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[1]Abstracted from a thesis presented to the Faculty of Washington University, in candidacy for the degree of Master of Arts, June, 1911.

(a) A temporary decrease in total number of bacteria present, with a subsequent large increase: Krüger and Heinze (3), Massen and Behn (4), Pfeiffer (5), Hiltner and Störmer (6), Russell and Hutchinson (1), Hutchinson (7), and Fred (2).

(b) An increase in oxidation: Fischer (8), Hesselink von Suchtelen (9), and Darbshire and Russell (10).

(c) An increase in ammonification: Störmer (11), Scherp (12), Laidlow and Price (13), Lipman (14), and Russell and Hutchinson (1).

(d) A detrimental effect upon nitrification: Warington (15), Scherp (12), Laidlow and Price (13), Perraud (16), Pfeiffer (5), Wagner (17), and Russell and Hutchinson (1).

(e) A beneficial effect upon nitrification: Brailles (19), Wollney (20), Pagnoul (21), Störmer (11), Hiltner and Störmer (6), Coleman (22), Lipman (14), Koch (23), and Fred (2).

(f) A detrimental effect upon nitrogen fixation: Störmer (11), Koch (23), Massen and Behn (4), and Koch and associates (24).

(g) A beneficial effect upon nitrogen fixation: Kramskii (25), Heinze (26), Lipman (14), and Fred (2).

(h) A detrimental effect upon denitrification: Wagner (17), Hiltner and Störmer (6), Störmer (11), Lipman (14), and Fred (2).

(i) In regard to the effect upon nodule organisms: Wollney (20), and Nobbe and Richter, believed such beneficial, while Perrotti (28), Koch (23), and Fruwirth (29) held to the opposite view.

In addition to the above, Hiltner and Störmer, and Russell and Hutchinson have studied with some care the flora, as a whole, prior and subsequent to treatment; and they found that certain types were detrimentally and others indirectly beneficially affected. Russell and Hutchinson have also studied, more or less, the effect of such treatment upon the micro-fauna and, as a result, claim that all types of animal

life, with perhaps one exception, are totally destroyed. Fred, in addition to his study with soils, studied certain types of soil organisms in liquid cultures and found that the addition of certain strengths of various antiseptics stimulated development. This strength varied with different organisms and the stimulative effect diminished gradually from the maximum in both directions.

EXPERIMENTAL.

The work outlined in this paper has to do primarily with the effect of CS_2, toluol, and, to a less extent, chloroform, upon the number of bacteria and the number of types of animal life before and after the application, in varying quantities, of such substances to soils.

EFFECT UPON THE NUMBER OF BACTERIA.

In studying the effect of such chemicals upon the number of bacteria in soils, the following methods were employed: the sample of soil to be studied was carefully freed from worms, etc., and sieved to either remove or break up all large particles, then thoroughly mixed. Sufficient water was added with that already present to bring the content up to one-third or one-half saturation, usually one-half. The desired quantity was then weighed into a sterile dish, care being taken to prevent contamination. The desired quantity of chemical was then added and mixed in; the dish was covered with a glass to prevent evaporation and set aside at room temperature for the desired length of time. In some experiments the treated soil was removed to sterile bottles, in which case the bottles were tightly stoppered with cork or even sealed. No differences were to be noted following these slightly different methods. The per cent of chemical added was in every case based upon dry weight of soil—dried at 110° C. for two hours.

Analyses of all soils were made as follows: the complete sample of soil was thoroughly mixed with a sterile spatula and several small portions taken from different parts of

the dish. This composite sample was again thoroughly mixed and several smaller quantities removed, and from this lot, after a second thorough mixing, the final weighing was made. Weighing was done on analytical balances, a sterile piece of paper being placed upon the pan; and in every instance, one gram of soil was taken. The gram of soil was at once removed to a 125 cc. rubber-stoppered Erlenmeyer flask containing 99 cc. of sterile water. This was shaken vigorously three times for one-third minute each, and while still in motion 1 cc. removed, by means of a sterile pipette, to another water blank. This process was continued until the sample was so dilute that a Petri dish containing 1 cc. could be counted. Duplicate platings were made of the final dilution, using approximately 8 cc. of agar. At first, standard nutrient agar + 1 was used, but this was soon replaced with the synthetic agar proposed by Lipman. Cultures were incubated at room temperature until maximum counting development was attained. With nutrient agar the period was usually three days; with Lipman's synthetic agar one week. Counting was done with a x10 hand lens; and if duplicates varied widely, they were discarded. Only averages are given.

In all, twenty samples of soil have been subjected to one or all of the three chemicals mentioned for periods varying from one day to six weeks. The samples represent soils from rich garden, orchard, forest, lawn, greenhouse, and pasture.

Table I contains a summary of all samples tested, while Table II contains a very conservative illustration of actual results. Table III contains the results of seven samples analyzed after being subjected to different quantities of CS_2 and toluol for three days. Table X contains complete results of the twenty samples tested.

TABLE I.

SHOWING THE NUMBER OF SAMPLES TESTED WITH VARYING STRENGTHS OF TOLUOL, CS_2, AND CHLOROFORM AND THE NUMBERS GIVING INCREASE, DECREASE, AND NO CHANGE IN BACTERIAL CONTENT.

	Toluol				CS_2				$CHCl_3$			
%	S.	S.I.	S.D.	?	S.	S.I.	SD.	?	S.	S.I.	S.D	?
.01	5	4	1	0	5	2	0	3	4	1	0	3
.1	5	3	0	2	5	4	1	0	4	2	0	2
.2	9	8	0	1	7	7	0	0	....	....	....	....
.3	1	1	0	0	1	1	0	0	....	....	....	....
.4	1	0	1	0	2	2	0	0	....	....	....	....
.5	1	1	0	0	1	0	1	0	....	....	....	....
1.	13	5	8	0	13	5	7	1	7	1	6	0
4.	6	2	4	0	6	3	3	0	....	....	....	....
5.	5	0	5	0	3	0	3	0	3	1	2	0
10.	7	0	7	0	7	0	7	0	7	0	6	1
20.	2	0	2	0	2	0	2	0	2	0	2	0

S. = samples tested. S. I. = samples showing an increase.
S. D. = samples showing decrease. ? = samples showing no change.

TABLE II.

SHOWING THE EFFECT OF TOLUOL, CS_2, AND CHLOROFORM UPON THE NUMBER OF BACTERIA IN A SOIL WHEN EVAPORATION WAS PREVENTED.

	Toluol				CS_2				$CHCl_3$			
%	1d.	3d.	7d.	42d.	1d.	3d.	7d.	42d.	1d.	3d.	7d.	42d.
Chk.	3.8	4.7	8.8	3.4	3.8	4.7	8.8	3.4	3.8	4.7	8.8	3.4
.01	5.5	8.1	14.3	6.6	7.5	8.5	7.3	4.7	3.8	6.2	5.8	2.9
.1	3.7	4.5	28.0	14.4	4.3	6.4	4.7	1.5	3.5	5.5	4.9	4.4
1.	2.4	1.8	3.0	2.4	1.9	3.6	5.6	5.5	2.4	2.7	3.3	2.0
10.	2.2	2.6	2.1	1.3	2.0	1.9	2.5	1.5	2.4	2.6	2.2	1.8
20.	1.6	1.4	2.9	1.1	2.0	2.3	2.2	2.5	1.9	2.5	2.4	1.9

Figures=million bacteria per gram soil.
1d., 3d., etc.=days after treatment.

TABLE III.

SHOWING THE NUMBER OF TYPES OF PROTOZOA AND NUMBER OF BACTERIA AFTER SUBJECTING SAMPLES OF SOIL TO TOLUOL AND CS_2 FOR THREE DAYS.

	Types Protozoa Present							Bacteria — Million Per Gram Soil						
Treatment	Chk.	T. .2%	T. 1.%	T. 4.%	C. .2%	C. 1.%	C. 4.%	Chk.	T. .2%	T. 1.%	T. 4.%	C. .2%	C. 1.%	C. 4.%
Soil S. 1....	7	3	0	0	6	5	0	2.0	2.0	2.0	1.6	2.8	1.4	1.0
" " 2....	7	7	....	3	8	....	5	13.2	23.2	.....	31.3	16.3	.....	7.6
" " 3....	7	7	....	4	10	....	6	14.8	22.8	.....	2.8	15.6	.....	23.2
" " 4....	9	9	1	1	9	7	1	5.4	7.3	30.8	4.5	6.5	8.4	19.6
" " 5....	6	5	0	0	4	2	2	11.4	14.4	15.2	40.0	16.3	17.0	8.6
" " 6....	6	7	2	0	7	5	5	5.0	17.4	7.1	4.8	12.4	62.5	27.0
" " 7....	8	*7	7	....	*8	5	....	13.9	*42.0	8.6	.....	21.1	*34.5	....
Total Types	12	11	7	4	12	10	6	.....	.....	.....	.....	.....	.....	....

T. = toluol; C. = CS_2; S. = sample.

*By error .3% was added.

The principal facts brought out in these four tables may be summarized as follows:—

(1) The chemicals studied when applied to soil in small quantities exert a stimulative effect upon the multiplication of aerobic bacteria, capable of developing upon nutrient agar or Lipman's synthetic agar.

(2) This stimulative effect may make itself markedly evident as early as twenty-four hours after treatment, and may remain evident as long as forty-two days—the longest trial period—even when evaporation is prevented.

(3) This stimulative effect is markedly evident with .01%—the weakest trial quantity—and may be evident with 4%, there being apparently a wide variation with different soils.

(4) The greatest stimulative effect lies apparently near .2% for the majority of soils tested, and beyond 1% we usually get a decrease.

(5) The decrease, when evident at 1.%, is nearly as marked with that amount as with 20%.

The above results were at such variance with those obtained by Russell and Hutchinson, the only persons, so far as the writer is aware, that have made laboratory investiga-

tions, even when their work was duplicated as nearly as possible, that a cause was looked for in the period at which analyses were made. While in their work it is not stated whether analyses were made before or after evaporation of the chemical, presumably it was after. To determine this point an experiment was made which gave the following results:—

Check not evaporated .....10.2 million.
.2% Tol. not evaporated.....41.6 "
.2% " after evaporation....00.56 "

As to whether this is the explanation, the writer is unable to say.

Russell (30) further states that "many if not all the active forms of bacteria are also killed" by the treatment. To determine whether this were true, even when a decrease was noted, analyses of a sample were made after treatment, but before evaporation, and again after evaporation. In the evaporation of this sample, the water content fell from 10% to 3%, and in Table IV is given the percentage decrease due to this loss of water.

TABLE IV.

SHOWING THE PERCENTAGE REDUCTION IN NUMBER OF BACTERIA AND ACTUAL REDUCTION IN TYPES OF PROTOZOA DUE TO EVAPORATION OF CHEMICAL AND MOISTURE.

Treatment	% Decrease of Bacteria	Protozoa Present	
		Before	After
Check	48	6	7
CS_2 0.2%	68	6	5
CS_2 1.0%	60	4	3
CS_2 4.0%	72	0	0
Tol. 0.2%	61	3	3
Tol. 1.0%	..	0	0
Tol. 4.0%	73	0	0

CS_2 1.%, CS_2 4.%, and Tol. 4% showed a decided decrease in number of bacteria during exposure to chemical.

If all vegetative cells were killed by the chemicals in question, a reduction of the per cent of water from 10 to 3 in the short space of 48 hours should not give us a decrease in number of organisms. From these results and conclusion (5) above, it seems evident, that even when there is a reduction it only affects certain species and not the vegetative cells of all species. This is in accord with the results of Hiltner and Störmer, and in part with those of Russell and Hutchinson in that they found quite a difference in the types of organisms subsequent to treatment.

Greenhouse Experiments.

In Table V is given an excellent and typical example of the effect of the substances studied on the number of bacteria in soils under greenhouse conditions.

TABLE V.

SHOWING THE BACTERIAL CONTENT OF A SERIES OF GREENHOUSE PLATS DURING THE GROWTH PERIOD OF A CROP, WITH THE RELATIVE YIELD OF THE SAME.

Date	2-21	2-25	3-4	3-11	3-18	4-3	4-18	5-10	Total Yield	Grain Yield
Chk.	.81	.63	5.50	4.60	1.60	2.70	4.10	2.00	100	100
Sterilized	00	.....	130.00	174.00	106.00	102.00	50.00	83.00	155	213
Pt. St.	.12	1.90	39.00	91.00	42.00	106.00	141.00	116.00	209	259
T. 22.5cc.	.81	.74	2.40	2.50	.74	3.40	4.00	4.00	102	96
T. 62. cc.	.81	1.50	3.10	1.00	2.10	4.00	2.20	2.00	123	158
T. 310. cc.	.81	.75	5.40	5.40	2.10	6.70	7.10	2.70	141	180
Chk.	.81	.63	5.50	1.60	2.40	2.20	3.30	1.20	100	100
Sterilized	00	.....	130.00	140.00	74.00	163.00	159.00	35.00	79	36
Pt. St.	.12	1.90	39.00	68.00	22.00	86.00	74.00	38.00	136	68
CS_2 22.5cc.	.81	1.30	3.00	.93	.85	4.40	4.20	2.00	113	119
CS_2 62. cc.	.81	.93	.70	1.70	.82	3.90	2.90	1.80	144	150
CS_2 310. cc.	.81	.73	3.90	1.50	.68	3.50	5.80	6.70	73	80
$CHCl_3$ 62. cc.	.81	.....	1.00	.53	1.00	2.60	2.90	.....	.....	.....

Figures on left of double line = bacteria in million; those on right relative yield. Plats above line seeded to oats, those below to buckwheat. T. = toluol. Pt. St. = partially sterilized.

This experiment consisted of twenty-five plats 1 x 3 feet in size and six inches deep. They were filled with a pasture soil (as poor as could be had) and treated in fours,

with the exception that only one was treated with chloroform. The soil in four plats was sterilized in autoclav (tests showing sterilization to be complete). Four were filled with soil partially sterilized, *i. e.,* heated to a temperature of 90°—100° C. in autoclav without pressure. The chemicals were added in three holes at equal distances apart and in three strengths: 22.5 cc. approximating 7 cc. per square foot or a weak practical application, however, one with which other experimenters have obtained marked increases in yield; 62 cc. or approximately .2% of dry weight of soil; and 310 cc. or approximately 1.% dry weight of soil. After allowing the chemicals to act for four days, the plats were thoroughly stirred; twelve were seeded to oats and thirteen to buckwheat. Duplicate plats were separated as far as possible.

Three samples were taken from the whole depth of duplicate plats by means of sterile tin cylinders. These samples were then thoroughly mixed and analyses made as previously given. Table V gives the bacterial count at intervals, from beginning until just before harvest, also the relative yield of duplicates, both in total dry weight and seed. There were some slight variations in the water content at times, but this was not sufficient, in any of the plats treated, to cause a difference in bacterial content.

From this experiment, typical of others and selected because of the more marked increase in yield and wider variation in treatment, but one conclusion can be drawn: *That with this soil no increase in bacterial content was evident with the wide variation in applications, but, at the same time, a marked increase in yield was obtained.*

With heat, the results were different. There was an enormous increase in number of bacteria, not, however, necessarily concomitant with an increase in yield. In the case of this soil, there were evidently toxic substances produced in the process of heating, for most of the young oat plants died as a result, and those that did not, made no growth for several weeks. With buckwheat, the plants were unable to overcome this detrimental effect in time to show

beneficial results. Similar toxic effects have been reported by Lyon and Bizzell (31), Schulze (32), and Pickering (33).

TABLE VI.

SHOWING THE PRESENCE OF DIFFERENT TYPES OF PROTOZOA AND NUMBER OF BACTERIA FOLLOWING TREATMENT OF GREENHOUSE PLATS.

Treated	Types Protozoa Present 12-15-'11												Bacteria—Million Per Gram Soil	
12–7–'11	a	b	c	d	e	f	g	h	i	j	k	Total	12–15	12–29
Check.....	+	+	+	+	+	+	+	+	—	+	—	9	9.0	7.0
CS_2 37cc.....	+	—	+	+	+	+	+	+	—	+	—	8	13.6	11.6
Tol. 37cc. ...	+	+	+	+	+	+	+	+	+	—	—	9	10.8	11.2
CS_2 350cc. ..	+	—	+	+	+	+	+	+	+	—	—	8	17.4	15.7
Tol. 350cc...	+	+	+	+	+	+	+	+	+	—	—	9	13.7	19.5

+ = present; — = absent.

In Table VI is given another series of greenhouse plats in which soil was taken from an uncultivated orchard and was by no means a poor soil. The weaker application represents approximately 10 cc. per square foot, while the heavier represents approximately 100 cc. per square foot, or .3%. In both cases the antiseptic was mixed in the soil when applied. Here we have an unmistakable initial stimulative effect on number of bacteria. These plats are still under observation.

From these results the conclusion seems justifiable: *That under the experimental conditions given above the chemicals studied in quantities equivalent to 100 cc. per square foot, or 1.%, do not exert a diminishing effect upon the number of bacteria, but may or may not exert a stimulative effect.*

Field Experiments.

About May 15, 1911, four series of plats (S. I, S. II, S. III, and S. IV), comprising a total of 140 plats, were laid out in the Missouri Botanical Garden. Each plat measured 5 x 10 feet and was surrounded by a trench one foot wide

and five inches deep. The plats in S. I, S. II, and S. III, were treated in duplicates of seven, as follows: check, toluol 1 cc. per square foot, toluol 15 cc. per square foot, CS_2 1 cc. per square foot, and CS_2 15 cc. per square foot. S. IV was subdivided into three series and treated as follows: *(a)* in duplicate, check, toluol 1, 5, 10, 15, and 30 cc. per square foot; *(b)* singly, check, CS_2 1, 5, 10, 15, and 30 cc. per square foot; *(c)* in triplicate, check, toluol 1 and 15, and CS_2 1 and 15 cc. per square foot. The chemicals were added in one hole per square foot punched to the depth of five inches.

S. I was seeded to oats; S. II to buckwheat; S. III to corn; and S. IV to millet.

Unfortunately, no examinations of soil for bacteria were made until August 19, hence the initial effects are unknown. Table VII contains the results of such examinations as were made. Samples for these analyses were taken two or three in number from each duplicate plat and treated, as previously given.

TABLE VII.

SHOWING THE NUMBER OF TYPES OF PROTOZOA AND NUMBER OF BACTERIA IN FIELD PLATS THREE TO FOUR MONTHS AFTER TREATMENT.

Types of Protozoa Found

Soil Treated 5–15–'11	Tol. cc. Per Sq. Ft.						CS_2 cc. Per Sq. Ft.					
Sampled	Chk.	1	5	10	15	30	Chk.	1	5	10	15	30
S. II 8– 9–'11...	9	8	...	...	9	...	.....	9	...	...	9	...
S. III 7–28–'11...	6	6	...	...	8	...	.....	6	...	...	7	...
S. IV 8–31–'11...	7	7	8	8	9	8	8	7	7	7	7	7
S. IV 9–11–'11...	9	8	8	8	9	8	9	9	7	8	9	7
Total No. Types..	9	8	8	8	9	8	9	9	7	8	9	7

TABLE VII—Continued.

Number Bacteria Per Gram Soil

Soil Treated 5–15–'11	Tol. cc. Per Sq. Ft.						CS_2 cc. Per Sq. Ft.					
Sampled	Chk.	1	5	10	15	30	Chk.	1	5	10	15	30
S. II 8– 9–'11..	8.1	8.9	...	...	9.2	....	8.1	9.6	...	....	9.6	....
S. III 7–28–'11..	10.6	10.8	...	...	12.7	....	10.6	8.8	...	....	8.4	....
S. IV 8–31–'11..	6.8	9.2	8.5	8.2	9.0	10.5	7.9	10.8	7.8	15.1	10.9	11.3
S. IV 9–11–'11..	6.8	7.7	7.5	7.7	5.0	7.7	5.8	4.2	9.9	9.2	6.6	2.7

S. IV samples include both *(a)* and *(b)*.

In addition to the above, a series of small plats of the same soil were treated to determine the first effects of the treatment. The quantities added with record of examinations made are given in Table VIII.

TABLE VIII.

SHOWING THE NUMBER OF TYPES OF PROTOZOA AND NUMBER OF BACTERIA IN FIELD PLATS FOLLOWING TREATMENT.

Treated 7–26–'11	Types Protozoa			Bacteria Per Gram	
Treatment	8-31 '11	9–7 '11	Total	8–31 '11	9–7 '11
Check.....	6	9	9	13.8	16.8
T. 10cc. per sq. ft.....	6	7	7	32.4	14.4
T. 50cc. " "	7	6	7	32.4	50.6
Check.....	4	8	8	14.9	13.3
CS_2 10cc. per sq. ft.....	6	6	7	89.0	18.1
CS_2 50cc. " "	7	6	7	14.2	16.2

Figures for bacteria equal million.

A third series was treated on the College Farm, University of Missouri, and a record of treatment together with results of examinations made thus far is given in Table IX.

TABLE IX.

SHOWING NUMBER OF TYPES OF PROTOZOA AND NUMBER OF BACTERIA IN FIELD PLATS FOLLOWING TREATMENT.

Plats Treated 11–8–'11	Tol. cc. Per Sq. Ft.					CS_2 cc. Per Sq. Ft.				
Sampled	Chk.	1	10	25	50	Chk.	1	10	25	50
				Types of Protozoa Present						
11–15–'11	9	7	9	9	9	9	6	7	8	7
12– 1–'11	11	11	10	9	7	12	10	11	6	8
12–14–'11	11	10	9	9	8	9	10	7	7	8
				Bacteria Per Gram Soil						
11–15–'11	14.0	14.2	18.9	16.8	.....	14.0	16.0	15.6	10.0	13.3
12– 1–'11	15.6	14.2	18.0	18.6	15.3	19.2	14.3	14.2	.....	39.9
12–14–'11	5.2	4.9	6.4	7.3	23.5	6.0	5.4	5.7	6.7	6.8

Figures for bacteria equal million.

From these results the conclusion seems justifiable: *That while the chemicals used may exert a slight stimulative effect upon the multiplication of bacteria subsequent to treatment, all effects of this action were, in the soil studied, lost within three months time.*

TABLE X.

SHOWING THE NUMBER OF BACTERIA IN TWENTY SAMPLES OF SOIL AFTER BEING SUBJECTED TO VARYING QUANTITIES OF TOLUOL, CS_2, AND CHLOROFORM FOR VARYING PERIODS OF TIME.

Sample	1	2	3	4	5	6	7	8	9	10	10	11	12	13	13	14	14	14	14	15	15	16	17	18	19	20
Days Treated	3	3	3	3	3	3	3	3	3	1	7	3	3	3	14	1	3	7	42	1	7	3	3	3	3	3
Chk.	6.6	5.4	14.0	13.2	14.8	11.4	5.0	2.0	66.0	79.8	53.9	84.0	27.5	1.4	1.2	3.8	4.7	8.8	3.4	5.1	6.3	9.8	7.4	6.9	6.7	3.4
Tol. .01%	....	....	....	....	....	....	....	....	.....	88.2	55.7	.....	....	1.2	1.0	5.5	8.1	14.3	6.6	....	....	....	....	....	12.9	....
Tol. .1 %	....	....	....	....	....	....	....	....	.....	92.4	79.0	.....	....	1.3	1.0	3.7	4.5	28.0	14.4	....	....	....	....	....	18.7	....
Tol .2 %	10.3	7.3	....	23.2	22.8	14.4	17.4	2.0	103.0	.....	.....	.....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	9.8
Tol .3 %	....	....	42.0	....	....	....	....	....	.....	.....	.....	.....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Tol. .4 %	....	....	....	....	....	....	....	....	.....	.....	.....	.....	14.0	....	....	....	....	....	....	....	....	....	....	....	....	....
Tol. .5 %	....	....	....	....	....	....	....	....	.....	.....	.....	.....	....	....	....	....	....	....	....	....	....	....	....	....	13.7	....
Tol. 1. %	....	30.8	8.9	....	....	15.2	7.1	2.4	.....	6.7	47.0	.....	....	0.9	1.1	2.4	1.8	3.0	1.8	2.4	2.2	4.8	3.4	....	12.6	....
Tol. 4. %	....	4.5	....	31.3	2.8	40.0	4.8	1.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	....	.....	....
Tol. 5. %	....	....	....	....	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.8	3.2	2.9	3.5	....	3.4	....
Tol. 10. %	....	....	....	....	....	....	....	....	.....	5.9	5.0	.....	....	1.0	0.6	2.2	2.6	2.1	1.3	2.6	2.5	1.8	3.3	....	.....	....
Tol. 20. %	....	....	....	....	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	1.6	1.4	2.9	1.1	.....	.....	.....	.....	....	.....	....
CS_2 .01%	....	....	....	....	....	....	....	....	.....	80.0	49.1	.....	....	1.3	1.2	7.5	8.5	7.3	4.7	....	....	....	....	6.7	.....	....
CS_2 .1 %	....	....	....	....	....	....	....	....	.....	119.3	61.3	.....	....	1.8	1.7	4.3	6.8	4.7	1.5	....	....	....	....	5.3	.....	....
CS_2 .2 %	....	6.5	....	16.3	15.6	16.3	12.4	2.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	5.8
CS_2 .3 %	....	....	21.1	....	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	....
CS_2 .4 %	....	....	....	....	....	....	....	....	.....	.....	.....	104.0	54.0	....	....	....	....	....	....	....	....	....	....	....	.....	....
CS_2 .5 %	....	....	....	....	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	5.1	.....	....
CS_2 1. %	....	8.4	34.5	....	....	17.0	62.5	1.4	.....	37.1	57.1	.....	....	1.6	5.0	1.9	3.6	5.6	5.5	4.9	5.4	5.0	5.8	5.2	.....	....
CS_2 4. %	....	19.6	....	7.6	23.2	8.9	27.0	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	....
CS_2 5. %	....	....	....	....	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.3	1.8	7.3	3.8	....	.....	....
CS_2 10. %	....	....	....	....	....	....	....	....	.....	3.5	2.8	.....	....	0.8	0.6	2.0	1.9	2.5	1.5	2.3	2.2	6.7	3.4	....	.....	....
CS_2 20. %	....	....	....	....	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	2.0	2.3	2.2	2.5	.....	.....	.....	.....	....	.....	....
$CHCl_3$ .01%	....	....	....	....	....	....	....	....	.....	77.7	50.6	.....	....	1.4	3.7	3.8	6.2	5.8	2.9	....	....	....	....	....	.....	....
$CHCl_3$ .1 %	....	....	....	....	....	....	....	....	.....	92.4	48.0	.....	....	1.4	1.2	3.5	5.5	4.9	4.4	....	....	....	....	....	.....	....
$CHCl_3$ 1. %	....	....	....	....	....	....	....	....	.....	6.7	44.0	.....	....	1.0	.9	2.4	2.7	3.3	2.0	2.5	6.0	11.3	3.3	....	.....	....
$CHCl_3$ 5. %	....	....	....	....	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.1	2.1	12.3	2.5	....	.....	....
$CHCl_3$ 10. %	....	....	....	....	....	....	....	....	.....	2.8	39.0	.....	....	1.0	.8	2.4	2.6	2.2	1.8	2.6	1.8	9.5	2.2	....	.....	....
$CHCl_3$ 20. %	....	....	....	....	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	1.9	2.5	2.4	1.9	....	....	....	....	....	.....	....

Figures = million per gram moist soil.

EFFECT ON PROTOZOA.

The casual observation of the abundance of certain types of protozoa in cultures of *Azotobacter* from soils previously "partially sterilized," led the writer to undertake a series of experiments to determine whether subjecting local soils to the treatment described by Russell and Hutchinson actually freed them of protozoa. This series of experiments has brought forth some very startling and interesting results, compared with the results of the above mentioned writers.

The same soils as mentioned under the bacteriological part of this paper were also investigated as to protozoa. The methods used were as follows, and while they were by no means satisfactory, they are the best the writer has been able to devise, and do reveal certain facts that seem worthy of mention:—

Samples of treated soil were mixed with twice their weight of sterile water or culture medium in an Erlenmeyer flask, thoroughly shaken, and set aside at room temperature to incubate. At first 20 grams were used, later 50 grams. In the first work, only water cultures were made, but it was later found that if a culture medium, composed of 1% hay decoction (1% hay infusion heated one-half hour in Arnold sterilizer, filtered and sterilized) and 5% white of egg, was used, certain types of protozoa, notably *Amoeba, Colpoda,* and certain flagellates that escape observation in water cultures, would develop in large numbers. No doubt if other media were employed other types would be observed. Now every sample is cultured both in water and in the medium given above.

After incubating the water cultures for twenty-four hours, 5 or 10 cc. of the supernatant liquid were removed (without stirring) with a sterile pipette to a sterile centrifuge tube. The centrifuge was then run until experience had shown practically all protozoa to be thrown down; 1 to 5 minutes usually sufficed. This can be determined by examining the upper portions of the liquid. A sterile pipette was inserted to bottom of tube and sediment removed and

examined microscopically. If examined earlier than twenty-four hours, the particles of soil held in suspension interfered with the examination. Examinations were continued at intervals for at least a week. It was found useless to examine the cultures in media under one week's incubation as the same types appeared there as in water cultures. After the first week such cultures were examined at intervals for two weeks. Often the cultures in media were so overrun with one or two types that development of other types must have been inhibited. A summary of results obtained thus far is given in Table III.

Where a "type" is spoken of, it simply means that those organisms were so different morphologically that they could be easily distinguished under the microscope from the other "types" observed. In some instances the term "type," as here used, undoubtedly includes two or more species. Some of the more common types appearing have been recognized as *Amoeba, Colpoda, Vorticella, Strombidium,* and *Dileptus.* In some instances, a rotifer and nematode are included, but since these organisms do not encyst they should be more sensitive to such substances, hence their inclusion should not invalidate the results obtained. The letters *a-b,* etc., in Table VI refer to "types" and show their presence following different treatment.

From this it appears: (1) *That in the seven soils studied CS_2 and toluol in a strength of .2% do not exert a diminishing effect upon the number of types of protozoa present;* (2) *That in strengths of 1.% and 4.% the chemicals studied do exert a slight diminishing effect upon the number of types present, but that several types (7 with toluol 1.%, 4 with toluol 4%, 10 with CS_2 1.%, and 6 with CS_2 4.%) are able to withstand these strengths.*

Greenhouse Experiments.

The same samples taken from greenhouse plats as referred to in Table VI, taken eight days after treating, were also examined for protozoa and the results of this examination are recorded in the same table.

From this we can draw but one conclusion, *i. e., That with the soil studied under the conditions of this experiment, quantities of CS_2 and toluol equivalent to 100 cc. per square foot, or .3%, even when stirred in, did not, within the first eight days, exert any influence upon the number of types of protozoa observed in the soil.*

Field Experiments.

The same samples of soil that were collected for bacteriological examinations from the three series of field plats given in Tables VII, VIII, and IX were also examined for protozoa and the results of such examinations are recorded in the same tables.

From these results the conclusion that *CS_2 and toluol, in quantities of 50 cc. per square foot when applied to the soils under study, had no diminishing effect upon the number of types of protozoa present* seems permissible. Such decreases as may appear in certain instances are well within the limit of error with the methods used.

GENERAL DISCUSSION.

In endeavoring to correlate the results just given with those previously reported along the same lines, certain difficulties are encountered. First, in regard to the initial effect of an application of the chemicals studied. Hiltner and Störmer, and Russell and Hutchinson have reported a very marked decrease, the former in field, and the latter in laboratory experiments, while the writer, applying the same quantities, has always noted either no effect or an increase in the number of bacteria present. The same investigators noted a subsequent enormous increase, while the writer has been unable to detect an increase that could not be attributed to the stimulative effect of the substance, unless such were added in amounts far exceeding those reported as used. If, however, in laboratory experiments quantities sufficiently large be used, such a decrease, followed by the subsequent increase, will be had. As pointed out

earlier, this difference, at least so far as initial effect is concerned, may be due to the time at which analyses were made, the process of evaporating the substance having been shown to decrease the number of bacteria from 50 to 75 per cent. The apparent correlation between the quantity of chemical required to give a marked decrease in protozoa and the quantity required to give a subsequent increase in bacteria would appear to support the theory of Russell and Hutchinson. This supports with equal strength, though, the theory of Greig-Smith (34) for an increase in quantity, within limits, would effect an equally increasing solution of toxic substances.

So far as the writer is aware, no one has reported a stimulative effect of small quantities of such chemicals (acting in soil) upon number of bacteria, but in the light of Fred's recent work such is not at all surprising. He has shown, beyond a doubt, the stimulative effect upon certain soil organisms when in a liquid medium. And while the quantities acting as a stimulant in soils are much greater than in liquids, in the light of the work of Lipman, and Stevens and Withers (35) this is not surprising, as they have shown that the activities of a complex soil culture in soils may be decidedly different from the activities of the same culture in liquid.

This stimulative effect may or may not be evident under greenhouse and field experimental conditions. If evident, the increase occasioned by it is soon lost. If the theory of Russell and Hutchinson be correct, the above contradictory results are easily explained, for in no case, under greenhouse and field experiments, have the chemicals studied exerted an appreciable effect on the protozoa, and in laboratory experiments, not with the quantities used (.2%) by these investigators (see Tables VI, VII, VIII, and IX).

Measuring the effect produced upon the total number of bacteria by the crop yield, greenhouse experiments have given negative results. In Table VI it is shown that, following applications far exceeding any field experiments thus far reported, no increase in bacterial content was

observed, while the increase in yield was as great as 80%. On the same soil, destruction of protozoa by heat followed by an enormous increase in bacterial content was not, in the case of buckwheat, followed by an increased yield. This may be attributed to the production of toxic substances, but, with oats, the toxic effect was even more marked at the beginning than with buckwheat, and still the increase in yield in one instance amounted to 159% on heated soil.

The writer's results show, as pointed out by Russell and Hutchinson, and Goodsey (36), that numerous types of protozoa are to be found in all soils examined. They do not show, though, that treating such soils with CS_2 and toluol, as has been done in experimental work or with the quantities used by the above mentioned writers (.2%), render such soils free from protozoa. To the contrary, they show no appreciable diminution in number of types present. This is true of laboratory, greenhouse, and field experiments. There is no doubt that sufficiently large quantities will destroy the protozoa, but such quantities are far beyond those of experimental practice. No explanation of why Russell and Hutchinson failed to observe protozoa following treatment can be offered, unless it be that their methods of examination were at fault.

The writer's results thus far regarding the effect of such treatment upon bacterial phenomena other than total number have been too meagre to report. However, the evidence of other writers seems to leave no doubt but that those activities which are of practical moment, namely: ammonification, nitrification, nitrogen fixation, and denitrification, are beneficially effected. (See Lipman, Russell and Hutchinson, and Fred.)

While protozoa have been shown to be universally present in soils, universally beneficial results do not follow the application of such chemicals. In the large series of plats (140), mentioned earlier in this paper, treated with amounts of CS_2 and toluol varying from 1 to 30 cc. per square foot, no beneficial results were observed with any test crops.

Similar negative results have been reported by many investigators.

Thus far, no method has been devised whereby the total number of protozoa can be determined; but, from observation of hundreds of samples, the total number of the different types seems to be very small in normal soils. The method used affords ample time for multiplication, and still the examination of 10 cc. of the supernatant liquid of a twenty-four-hour-old water culture (50 parts soil to 100 parts water) often reveals less than twenty-five organisms. When we take into consideration the low water content so often present in our soils, with the necessarily thin films of water and the small number of comparatively large organisms that must cover this soil, the assumption seems unfounded that such organisms prevent the multiplication of bacteria. In fact, computing the diameter of the theoretical water pore of a medium soil by Slichter's (37) formula, we find that for protozoa to pass through the soil they must pass through holes the diameter of which is many times less than the diameter of their bodies. While the writer does not wish to imply that it is in practice impossible for these flexible organisms to move about in the soil, he does wish to call attention to the fact that with the relatively few active organisms present, the physical conditions of the soil must render their coming in contact with a majority of soil bacteria highly improbable. Furthermore, the recent work of Goodsey speaks strongly against the existence in soils of protozoa in the active condition, at least under normal conditions. If this be true, their effect upon the bacterial content of the soil is nil. Under the conditions cited by Russell and Gadding (38), it seems possible that protozoa might become sufficiently numerous to produce an appreciable effect, but this is only an isolated abnormal condition.

After the body of this article had been written and conclusions drawn, the writer noted with a great deal of pleasure the following conclusions drawn by Emmerich, Leinigen and Loew (40), after investigating the same problem:

"So richtig auch Halli Thoris in vielen Fällen sein wird, so dürfte es doch nicht angänglich sein, dieselbe zu verallgemeinern."

GENERAL CONCLUSIONS.

From the evidence given above, the following conclusions appear justifiable:—

1. That small quantities of CS_2, toluol, and chloroform, such as have been used practically and experimentally, when applied to the soils studied, exert a stimulative rather than a diminishing effect upon the total number of bacteria present.

2. That an application of such quantities of CS_2 and toluol does not have an appreciable effect upon the number of types of protozoa present in such soils as have been studied.

3. That a very marked increase in yield may be noted following such an application when no evident change occurs in total number of bacteria present.

4. That, in the light of the recent work of Koch, Egoroo, Goodsey, Fred, and others, with results presented in this paper, the theory advanced by Russell and Hutchinson to account for the increased yield following the application of such chemicals, appears not tenable for general application.

The writer wishes to express his appreciation to Dr. William Trelease, Director of the Missouri Botanical Garden, who placed unlimited means at his disposal for carrying on this piece of work: to Dr. George T. Moore, at whose suggestion the work was undertaken and whose untiring directions have rendered the results obtained possible: and to Dr. W. C. Curtis, who placed, without reserve, his laboratory and equipment at the writer's disposal for carrying on the protozoalogical end of the work.

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THE RELATION OF ALGÆ TO DISSOLVED OXYGEN AND CARBON-DIOXIDE. WITH SPECIAL REFERENCE TO CARBONATES.

BY CHARLES OSCAR CHAMBERS.

INTRODUCTION AND REVIEW OF LITERATURE.

During the summer months of 1908 and 1909, while associated with the Puget Sound Marine Station at Friday Harbor, Wn., I undertook the study of some algæ growing in the tide pools and lower tide levels of Puget Sound. The study was suggested by Dr. T. C. Frye, Professor of Botany at the University of Washington, and sought to find an explanation for the great variations already noted by him, in *Prionitis lyallii* as found at different levels or exposed to different degrees of light, heat, and possibly to different amounts of dissolved gases.

My observations included a considerable range of conditions as shown along the shores of the different islands, with all possible degrees of exposure to light, air and tidal currents. There is a range of over ten feet from lowest to highest tide levels, and a range of 10° F. in temperature, viz., from 53° to 63° F.; the pools left at high tide or those filled by spray, being about ten degrees warmer than the main body of water, which is very constant at 53° F.

Here in these upper levels, in this warmer water, is the favorite haunt of the typical form of *Prionitis,* where it is smaller and more branched than at the lower levels. This was at first supposed to be an adaptation to a smaller supply of CO_2 in the warmer pools. The warmer water has a lower coefficient of absorption for the gases, oxygen and CO_2. The more branched and divided forms would present more absorptive surface to compensate for the smaller amount

of gas. Being more exposed to the sun than those much deeper in the water, the tougher, darker surface seemed to be a protective adaptation to these conditions, while the shorter, more compact and much-divided fronds could better resist the strain of the rough waves to which they are exposed in these higher positions. Whether the dark brown of the topmost plants, as opposed to the yellowish tinge of the lowermost ones, could be an adaptation to the different light intensities was not considered. The theory and explanation of Gaidukov ('04) and Blackman ('04) would lend some plausibility to such a view. Although *Prionitis* is left by the receding tide it is not required to resist drying out, as is the case in *Fucus* and its associates, for *Prionitis* is always covered by water, even in the highest pools. It is likely that there is some concentration of dissolved salts, due to evaporation, in such pools during low tide; but this, however, must be very slight in so short a time.

As it appeared on further investigation, however, none of the above explanations seemed adequate. The more luxuriant, though less typical growth, was found in places little exposed to strong light or increased temperature, and the best growth, with fronds least dissected, was found in a small bay where the water was the warmest and the exposure to sunlight the greatest. But this, like the other stations showing the most vigorous growth, had the advantage of constant currents to renew the supply of CO_2 and other foods, while affording a safe, protected position for attachment.

The fact, also, that young plants were always found starting around the rim of the pools where CO_2 would supposedly be abundant, but never on the bottom, where both oxygen and CO_2 would, in all probability, be absent or much diminished, though affording safer points of attachment, led to the belief that the whole problem would resolve itself into a question of aëration. The best growth was found at places best aërated, regardless of their position as to light, heat, or point of attachment, except as this might

afford protection from rough waves or currents. Where currents were most constant, if not too violent, bringing a supply of CO_2 and removing the excreted oxygen, an important factor in the growth of aquatic plants, there the largest growth was found, both as to size of individuals and total bulk of plant growth.

Lacking facilities at the time for measuring the gas content of the water, that point was necessarily left unsettled, and the problem, so far as that region is concerned, was dropped indefinitely. Some two years later, this phase of the problem was taken up at the Missouri Botanical Garden. Although the conditions are quite different in small bodies of fresh water from those just cited in larger bodies of salt water, yet Birge and Juday ('11) find results in Wisconsin lakes similar to those obtained by Lebedinzeff in the Caspian and Black seas, and the same problem, in general, presents itself for solution, viz.: what is the relation of dissolved gases to algæ or submerged aquatics; first, as to life and death, or continuity; second, as to periodicity or rate of reproduction; third, as to habit, or morphologic adaptation to environment?

Pfeffer ('00) remarks that currents of water are of the utmost importance in providing aquatic plants with continual supplies of dissolved carbonic acid. Owing to the marked solubility of the latter a given volume of water can hold a higher percentage than is present in the supernatant air, while still more may be held in the form of dissolved carbonates. Indeed, according to Hassak ('88), a green plant is not only able to obtain carbon-dioxide from calcium bicarbonate, $Ca(HCO_3)_2$, but also can make use of 70% of the carbon-dioxide contained in sodium bicarbonate. This agrees with the investigations of Darwin and Pertz ('96), who showed by laboratory experiments that in water that is kept stirred, photosynthesis is about twice as vigorous as in water that is allowed to remain quiet. This had to do only with the removal of excreted oxygen, whereby its accumulation in the plant tissues is prevented. This fact added to the benefits derived from a constant renewal

of CO_2 would be an important factor in the plant economy.

Dr. A. Disato ('08), after an investigation along the shore of the Mediterranean during the summer of 1907, says: "It is significant that the CO_2 content of the air over the sea and that over the land are the same (3 parts in 10,000), although the conditions are so different. Further, the CO_2 content of the sea air in the same region remains constant for a number of years by day as well as by night. He accepts the theory of Schloessing to account for the phenomenon. According to this, the CO_2 of the air and the carbonates and bicarbonates of the sea remain in equilibrium. The sea serves as a regulator of the CO_2 content of the air. By lessening the CO_2 pressure of the air, new carbonic acid comes from bicarbonates of the sea. On the other hand, by increased pressure, CO_2 is absorbed and more carbonates and bicarbonates are formed in the water. The constancy is further effected through transportation by the wind.

Dr. Disato made further investigations as to the oxygen content of shore waters. He found that the amount of oxygen dissolved in sea water at Concarneau fluctuated between 16 and 20 mg. or parts per million, or about 11 to 14 cc. per liter. This large amount is explained by the shallowness of the water along the coast and by the carpet-like colonies of algæ which cover the ground to a great extent in the shallow waters, and in sunlight are able to produce an abundance of oxygen. The best proof of this hypothesis is the fact that the amount of oxygen is greatest during long continued sunshine.

The extensive investigations of Whipple and Parker ('02) on ponds and reservoirs under their care, point to a very close relationship existing between the gas content of the water and the periodic growth of the phytoplankton in these waters. This is especially noted in the spring and autumn maxima of diatoms. The fluctuations in gas content are very much influenced by the movements of the water on a large scale, known as "overturning," at these times.

They say: "The relation of microörganisms to the oxygen content of waters have been investigated, but their relation to dissolved carbonic acid has received scant attention, probably because of the elusive nature of this gas in water and the difficulty of determining its amount. Inasmuch as the algæ and many of the troublesome protozoa are chlorophyll bearing, the relation is one of fundamental importance. All such organisms require both oxygen and carbonic acid in their metabolic processes.

"Nitrates are, indeed, important, but inadequacy of this explanation became manifest when it was observed that some water comparatively low in nitrates, at times supported large growths of algæ. Another reason for the development of the algæ in ground water suggests itself, namely, the presence of carbonic acid. Its occurrence in comparatively large amounts in waters of this class has been shown, and there is good reason to believe that the algæ are influenced by it more than by the nitrates, although the latter are necessary. The consumption of carbonic acid by diatoms has found illustration in several recent instances where *Asterionella* and *Synedra* have developed in ground waters exposed to the light. In the inlet of a certain reservoir where *Asterionella* was abundant, the water contained five to eight parts per million of carbonic acid, while at the outlet it was entirely absent."

Again, while the overturning brings up nitrogen as free ammonia to be oxidized to nitrates when it reaches a supply of oxygen, at the same time a supply of CO_2 is carried up by the same current which brings up the diatoms themselves. "It is quite possible that the duration of their growth is determined by the amount of CO_2 present, but observations on this point are not convincing."

In another article Whipple ('94) says: "That diatoms need air has already been shown by laboratory experiments; but whether it is the O_2 or the CO_2 gas of the air has not been determined."

In an earlier article Calkins ('92) shows that there is a maximum of diatoms in April and another in autumn,

reaching its climax in December. How much these depend upon gas content and how much on temperature and nitrogenous content is not fully established. Temperature is known to be an important factor.

Horation Parker ('11) found that *Synura* appeared in Lake Chochituate with a temperature of 48° to 50° F. and disappeared when the temperature fell below this. But he says: "Temperature is not the only factor that fostered the organisms at this place (the thermocline), for at higher levels (above the thermocline) there would have been less free carbonic oxide, and at lower ones less oxygen and less sunlight."

Marsh ('03) found *Synedra* in Green Lake, Wis., in nearly every month in the year, but in one winter especially it was flourishing from January to May. This fact, while difficult to reconcile with a part of Parker's theory, confirms his statement in another that the temperature is not the important factor. Fritsch ('07) also believes that the scarcity of diatoms in tropical waters is due to the small amount of dissolved gases in the water, rather than to an unfavorable temperature, though, in general, they prefer colder water.

Studies made by Whipple and Parker ('02) in previous years and also by Baldwin and Whipple ('06) furnish, in a degree, the lacking proofs and serve to confirm the conclusions of Parker as to the relative importance of these dissolved gases for phytoplankton in general. The studies of Marsh ('03) serve, also, in a way, to confirm this idea; for while Whipple and Parker ('02) found diatoms, such as *Synedra, Melosira* and *Cyclotella,* reaching their maximum in spring and summer, as Marsh did in Lake Winnebago, yet he finds it to occur in Green Lake in the depth of winter, for three successive winters. He quotes Voigt, also, as reporting the maximum for *Asterionella* as occurring in winter in Traumer-See and Edeberg-See. This would indicate that temperature is not the deciding factor.

Whipple and Parker ('02), in the studies above cited, describe a large growth of diatoms just below the thermo-

cline, the only place where an abundance of carbonic oxide occurred, while oxygen was to be found at all depths. At this time it was observed that above the thermocline the water was alkaline to phenolphthalein, indicating the presence of a certain amount of normal carbonates in solution. This alkalinity has been more frequently observed in connection with a vigorous growth of blue-green algæ. It is possible that certain organisms under certain conditions have the power of taking carbonic oxide from the hypothetical[1] bicarbonates of alkaline earths. This would accord with the facts noted long ago by one of the authors, that in Massachusetts water supplies, heavy growths of organisms were somewhat more likely to occur in hard waters than in soft waters. This fact has been observed since by a number of others, notably Wesenburg-Lund ('08) and Birge and Juday, to whom reference is made more fully later.

The cause of the sudden appearance of "water-bloom" is but little understood. Whether it is the cause of the changes noted in the alkalinity and gas content of the water, or whether it is the result of these conditions is still a question. Studies have been made to determine its danger to fish, etc., but scarcely anything is known as to the physiological relations to its environment. Steuer ('11) and others have claimed that by the rapid respiration at night of the plants comprising the "water-bloom," the water is robbed of so much dissolved oxygen that fish have been suffocated.

Baldwin and Whipple ('06) more recently report a case in Weequahic Lake, Newark, N. J., of a rapid growth of "water-bloom" followed by the sudden death of large quantities of fish, and attributed by them to the exhaustion of oxygen from the water. They remark that while green algæ are "powerful oxygenators" there is some reason to believe that the blue-green algæ are not so vigorous in this respect. It had been noted for a number of years that

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[1] Some of the alkaline earth bicarbonates have recently been prepared by Keiser and McMaster, Jour. Am. Chem. Soc. **30** : 1713-17. 1908, and Keiser and Leavitt, Jour. Am. Chem. Soc. **30** : 1717. 1908.

oxygen was deficient in reservoirs where the latter organisms predominated.

They found that in general the amount of CO_2 varied inversely with the amount of oxygen present. When the water became thoroughly mixed, through the action of the wind, there resulted, as a consequence, an even distribution of both the O_2 and the CO_2 throughout the vertical. When the amount of CO_2 was recorded as zero the water reacted alkaline to phenolphthalein, indicating normal carbonates. When the amount of CO_2 was recorded as minus it indicated the amount of CO_2 abstracted from the bicarbonates dissolved in the water. This power to abstract carbonic acid from the bicarbonates has been noticed, they say, by several observers, but no special attention has been called to the phenomenon. The exhaustion of oxygen from the water they attribute to the decay of the blue-green algæ; for, with the return of oxygen after the decay of the blue-green algæ, the grass-green algæ increased in amount and were believed to be the source of the excess of oxygen, which reached 149% of saturation. Stirring up the water and exposing different parts to the wind tends to reduce the amount of CO_2 in the water and at the same time increase the amount of oxygen in the water by aëration. If the circulation is a gentle one it may increase the amount of CO_2 by distributing throughout the mass that which is found at the bottom; but if the circulation is violent it may tend to decrease the CO_2 by losses into the atmosphere at the surface.

Morren ('41), who made an extensive study of "water-bloom," and especially that form known as red or bloody water, claims that the amount of oxygen and CO_2 varies reciprocally with the amount and kind of organisms found in the water; increasing during the day on account of the rapid photosynthesis of the green or reddish organisms, and diminishing in the night (the amount of oxygen) on account of the rapid respiration of the same organisms. Moreover, he claims that the color of these organisms is so much dependent on the oxygen content of the water that he can control the color by varying the supply of

oxygen, an increase producing an intenser red and a smaller amount resulting in green.

This explanation might account for the appearance of red *Euglena* in one of the fish ponds in the state hatchery, a little later than the appearance of the bloom on the other ponds. Some claim that such forms may change from holophytic to holozoic and *vice versa,* in a similar manner, along with change of food supply.

Kofoid ('08) reports the red *Euglena* as appearing in the Illinois River, always associated with *Euglena viridis* and, he thinks, possibly only a variety of the latter; but he does not seem to associate it with the variation in oxygen content of the water.

The sudden appearance of a red bloom on Jamaica Pond, near Boston, for a succession of summers, is reported by Mrs. Ellen H. Richards ('01), but it is not associated with any change in the water, except a rise in temperature. It consisted largely, if not wholly, of *Oscillatoria prolifica.*

The report of Birge and Juday ('11) on the dissolved gases of the Wisconsin lakes made its timely appearance after some ten or twelve years of mature study. This served to shed considerable light on the problem in hand.

In these studies they find much importance attaching not only to the free CO_2 brought by the convection currents, but likewise, and more especially, to the half-bound CO_2 contained in the bicarbonates of calcium and other alkaline earths. In fact these alkaline lakes support the richest algal flora and, consequently, the richest fauna. Lakes containing a large amount of these substances possess, according to them, a source of carbon-dioxide lacking in soft-water lakes and can, therefore, support a large population of plankton. The reduction of bicarbonates to mono-carbonates gives an alkaline reaction to the upper stratum of the lake. These mono-carbonates must take up the carbon-dioxide liberated in the upper water by respiration and decomposition, and more will be absorbed from the air than is possible if free carbon-dioxide is already contained in the water.

Angelstein ('10), working with a number of different kinds of water plants, found that photosynthesis, as shown by the bubble method, was proportional to the concentration of bicarbonates present in the water and that in distilled water, free from carbonates, but saturated with free CO_2, the photosynthesis was very slight. Water saturated with air alone gave very slight activity, as shown by the bubbles, but was greatly increased by the addition of a small amount of $KHCO_3$, or $NaHCO_3$. The maximum was reached at about 1% to 1.5% solutions, and above that decreased on account of too high osmotic pressure. Solutions of like osmotic pressure gave like results. Concentrated solutions of calcium bicarbonate always gave a higher oxygen excretion than tap water saturated with free CO_2. In natural waters an equilibrium is established between the CO_2 pressure of the carbonates in the water and the CO_2 pressure of the air.

An abundance of dissolved carbonates, according to Birge and Juday ('11), increases the supply of carbon-dioxide for plant use, both directly and indirectly; directly, by the original stock of half-bound CO_2 in the bicarbonates; indirectly, because the mono-carbonates take up more CO_2 from the air than would be absorbed without their aid, and also absorb that liberated in the upper water by respiration and decay. Much of this last would escape into the air, especially at night, if not so absorbed.

Morren ('41) confined a part of the water in an aquarium with its contained organisms, under a large balloon flask during the night. Analysis in the morning showed the gases present in a very different proportion from that found in the free water, twice as much CO_2 in the flask, showing that there must be a great loss of CO_2 from the free water during the night and presumably a like amount of oxygen during the day.

In such hard-water lakes as those above described, Birge and Juday ('11), find the *epilimnion* to be permanently alkaline during the summer, which shows that more CO_2 is withdrawn from the bicarbonates than is supplied to

them from other sources, and more than is made good at night. The process goes so far that mono-carbonates are precipitated to be redissolved by the free CO_2 in the lower water or accumulated in the mud at the bottom.

Davis ('01), in accounting for the origin of the marl in some of the small lakes in Michigan, gives the following:— after pointing out the inadequacy of animal life, evaporation and escape of CO_2 on relief of pressure, as causes of the precipitation of the carbonate of lime, the author discusses at length the only alternative hypothesis, namely, that the calcium salts are precipitated through the agency of plant life. In the marly lakes aquatic plants of all kinds become incrusted with calcium carbonate, which is not a true secretion of the plants, for it is purely external, and the same species in other districts are not incrusted.

If the calcium carbonate is in excess in the water, and is held in solution by carbon-dioxide, then the absorption of the latter by the plants causes precipitation of the carbonates upon those parts, stems and leaves abstracting the gas. But if the proportion of calcium carbonate in solution is so small that it would not be deposited even if there were no carbon-dioxide present, the precipitation is explained by the action of the oxygen set free by the living plants, in converting calcium bicarbonate to mono-carbonate:—$CaH_2(CO_3)_2 + O = H_2O + CaCO_3 + CO_2 + O$.

In lakes such as these the vigorous photosynthesis, thus made possible by this increased supply of CO_2 in an available condition, is shown by the resulting excretion of oxygen, which often exceeds the point of saturation. This we should expect to occur, since Dévaux ('89) has shown that the ratio, $\frac{CO_2}{O_2} = 1$, holds true for submerged aquatics as well as for aërial plants or leaves. Even in winter Knauthe ('99) found a much larger excess of oxygen in some of the ponds which he studied, when they were covered with ice. In one pond he found the surprisingly large amount of 49 cc. of oxygen per liter of water, just under the ice. This was about 515 per cent of the amount required for saturation. A phenomenal increase in oxygen was noted in this pond, the amount

at the surface rising from 29.8 cc. on January 16, noon, to 42 cc. on January 17, 12:30 P. M. This period of very high oxygen content lasted three days and was correlated with a very large growth of chlorophyll-bearing organisms. During the daytime, when the organisms were actively excreting oxygen, the water became alkaline as a result of the consumption of carbon-dioxide, presumably taken from the bicarbonates. It would be interesting to know the kind of organisms that were so active at so low a temperature, but this fact is not stated. The mantle of ice prevented the escape of oxygen, which often occurs during a strong wind in summer.

In some of the hard-water lakes studied by Birge and Juday ('11) the alkalinity reached a point where some of the normal calcium carbonate was precipitated out, thus forming a layer in which the combined CO_2 showed a pronounced decrease. Alkaline strata have been found in lakes which have very soft water. On the other hand, soft-water lakes, especially with very soft water, present very different conditions. No large supply of free CO_2 is readily obtainable, and since only a small quantity of half-bound CO_2 will be present in soft water, the total available CO_2 will be limited in amount. In general the soft-water lakes of Wisconsin, so far as observed, do not support so large a phytoplankton as the hard-water lakes, and no hard-water lakes have as yet been found which contained so little phytoplankton as many of the soft-water lakes.

Wesenberg-Lund ('08), in his comparative study of the fresh-water lakes of Denmark and Scotland, found that those of Denmark, which are nearly all shallow and usually alkaline, support a rich biota, while the lakes of Scotland, most of which are deep and acid on account of bogs and humic acid in the soil of the drainage basins, are very poor in plankton and submerged aquatics and, consequently, in the ordinary life of fresh-water lakes. The blue-green algæ which form so important an element in the littoral region of the Danish lakes, often covering the stones and depositing on them a layer of greenish lime, are almost non-existent in the corre-

sponding regions in Scotland. As regards the organisms themselves, the Cyanophyceæ play a very much less important part in Scotland than in Denmark, while conversely, the desmids are remarkably well represented in the plankton of the Scottish lakes and but feebly so in the Danish lakes, in common with nearly all central European lakes.

West ('04) notes that "Desmids thrive best in soft water, and they are most numerous in peaty water which has a trace of acid. With few exceptions they do not flourish in water containing carbonates of lime in solution, and no good collection of desmids can be made in a purely limestone district in which the water is hard."

Fritsch ('07) thinks that the scarcity of diatoms in tropical waters, noted especially at Ceylon, is due to the small amount of dissolved gases in the waters, and not to the high temperature, *per se,* except in so far as it influences the amount of dissolved gases. He found them only in aërated waters, *i. e.,* running streams. He did not have data as to the amount of dissolved gases, but assumes *a priori* that a high temperature, 25° C., must of necessity indicate a low gas content, as indicated by figures from Forel's tables. The lowest temperature attained by the lowland waters of Ceylon was 25° C., which in the table corresponds to 5 cc. of oxygen per liter.

	Temperature	Oxygen	CO_2	
Forel	5° C.	7.3 cc.	0.6 cc.	Per liter of water, as found in the waters of Lake Geneva.
	20° C.	5.7	0.3	
Roscoe and Lunt	5° C.	8.68		Standard.
	20° C.	6.28		Saturation.

In the fish-pond, mentioned as an example of temperate zone fresh water, near Bristol, England, Fritsch ('09) finds that diatoms play an important part. The free forms predominate during the winter months, but are rare during the summer months and occasionally absent. The water in this locality and latitude does not become very strongly heated,

and hence is not deprived of its dissolved gases, as is the case in the tropics just mentioned. However, the summer is marked by change of filamentous forms, such as *Melosira* and *Fragilaria.* It is believed that diatoms in general prefer colder and better aërated water, although Fritsch mentions the well-known cases of diatoms in hot springs, where they usually assume filamentous forms.

In this connection it is interesting to mention H. W. Clark's ('08) report of *Melosira* in Guatemala, where, he says, "One of the most striking features of the Amatitlan plankton is the abundance of *Melosira,* which is found abundantly in the bottom of nearly all the hauls, and usually makes up the main mass of all the filamentous material. The specimens occur in long rigid filaments."

West ('09) says: "Diatoms are not a feature of the plankton in the Yan Yean reservoir at Victoria, Australia, although *Melosira granulata* occurs abundantly and is never absent from the collections."

"After all," says Fritsch ('07), "We know as good as nothing at present as to the influence of the amount of dissolved oxygen in the water, on the various fresh water algal genera."

As to the desmids, both Fritsch ('07), in Ceylon, and West ('09), in Yan Yean, Australia, remarked the abundance and variety of and tendency toward the filamentous forms. West ('09) says that the most striking feature of the plankton at Yan Yean is the richness of its desmid-flora.

On the other hand, in the fish-pond near Bristol, Fritsch ('09) finds a notable lack of desmids, the number of individuals being small, and likewise the number of genera. This is no doubt due to the fact that water containing carbonated lime, which is known to be unfavorable for an abundant development of desmids, drains into the pond. This fact is also noted in regard to the absence of desmids from the calcareous waters of Kew and the Thames river. The desmids, which generally are so abundant and varied in the temperate waters, were found by Fritsch ('07) to be even more abundant and varied in the waters of Ceylon. The great variety of form, he says, may be only the result of a

longer evolution, but the predominance of filamentous forms in tropical waters, which are so rare in temperate waters, indicates an evident adaptation to poorly aërated waters, since they are found to be most abundant in small stagnant ditches and rock pools. Even the species which elsewhere exist as separate simple individuals are here found attempting a filamentous form. This is not merely a local condition, for in the upland pools (altitude 6,000 feet), where conditions are semi-temperate, the filamentous forms are just as rare as in the waters of temperate regions. He has elsewhere suggested that we may again find an explanation for these phenomena in the relative aëration of the waters, *the presence of little dissolved oxygen appearing to encourage filament formation.* This is a suggestion which must be left to experiment for verification, but it certainly would explain the relative abundance of filamentous desmids in the tropical waters. The same theory also will account for filamentous diatoms in the hot springs. In a foot-note it is stated that this theory is based on Senn's observations on colonial Protococcales.

Senn ('99) put *Cœlastrum* cells from pure cultures containing no cœnobia, into culture flasks containing nutrient solution. One flask he stirred up well with air so as to secure good aëration. The other flask was completely exhausted of air by means of a suction pump. After a week samples from the former showed only single cells, no cœnobia having been formed, while samples from the latter showed a number of eight and sixteen-celled cœnobia, perfectly green and with gelatinous conditions well developed.

In another case he put single cells into glass cylinders 12 cm. high, with 0.25% Knop's nutrient solution. Boiling the solution to sterilize it had driven out all the air. In a few weeks this contained cœnobia with gelatinous arms or connections developed. Similar cultures in 2% solutions, instead of 0.25%, gave similar results, showing that the action was not due to concentration of the solution. Moreover, distilled water gave the same results. The depth of the water prevented the diffusion of air to the lower layers of

water. From these experiments he concluded that *Cœlastrum* in well aërated water will form free cells, but in stagnant water will form cœnobia. He had gotten similar results from hanging drops of cane-sugar solution. Those which he stirred vigorously and mixed with air produced free cells only, while the unstirred solution formed cœnobia. The stirring had mixed in a considerable amount of air which had remained.

In order to test this further he arranged cultures in Erlenmeyer flasks (0.2% Knop's solution), likewise in open dishes. He boiled them to drive out all the air. After cooling, the flasks were closed and either oxygen or carbon-dioxide let in (manner not stated) to about half (½) pressure, kept air-tight under water, and shaken so as to mix as much as possible and saturate one solution with one respective gas (O_2 or CO_2). A little of the alga in four-celled colonies from pure cultures was then introduced by means of a curved pipette, and the flasks then closed air-tight with paraffine.

There were eight flasks in all; four with oxygen and four with carbon-dioxide. Two each were placed in the light and two each in the dark. After six days those with oxygen showed many free cells but no cœnobia. In the flasks with carbon-dioxide only a few of the 4-celled cœnobia had changed; but in two weeks many 8-celled cœnobia appeared. Those with O_2, left in the dark eight days, showed no change. Those with CO_2, left in the dark eight days, were developing daughter cells the same as those left in the light. Similar results were obtained with certain species of *Scenedesmus;* oxygen cultures yielded only single free cells, while cultures with CO_2 produced four-celled cœnobia when grown in tall 13 cm. cylinders. Likewise with *Dictyosphærium*; if the culture solution after sterilization was not shaken or aërated, many-celled cœnobia resulted—some even as high as 100-celled; while similar cultures at the same time, but shaken after sterilization, to secure aëration, yielded only single-celled colonies, or a few 4-celled to 16-celled ones. In the latter the cells stood farther apart, while in the former they grew more closely together.

PRELIMINARY TESTS.

As a preliminary step I made a series of determinations of the dissolved gases, oxygen and free CO_2, in the waters of the lagoon and stream of the Garden and in some of the ponds in the city parks, extending from May until the freezing of the ponds in January. The autumn and winter of 1911, up to January, were mild and most favorable for such studies. There was no severe freeze to kill or check the autumn growth, which was especially rich in diatoms. The summer was unusually warm; much of the time ranging from 90° to 100° F., from May to October. But this made only a slight difference in the supply of water in the ponds, which are fed from the City System, and an almost constant level is maintained, except when heavy rains cause a slight temporary rise and overflow at the outlet dam of the lagoon. The hot summer must have had some considerable influence on the temperature of the water, as it nearly always showed the same temperature as the air during the summer and early autumn. The chemical composition of the water fluctuates somewhat with the heavy rains, but aside from this it must be the same as that of the Mississippi River, from which it comes.

While the chemical value of the water as a culture medium for algæ is not materially changed by the process of purification at the city settling plant, yet there are slight changes that would have to be taken into account where chemical indicators are involved. Miss Hayden ('10), in her recent study of the algæ in these ponds, makes a general statement on authority, to this effect. The annual report of the Water Commission for 1906, the last available, shows that the process removes nearly all of the bicarbonate of calcium and delivers the water with quite a strong degree of alkalinity. This was a point with which I was later to reckon.

INVESTIGATIONS—FIELD.

The accompanying table of oxygen and carbon-dioxide content of the water in the lagoon shows an increase of oxygen

from 3.3 cc. per liter, June 23, to the saturation point of 7.80 on July 20, when the "water-bloom," consisting mostly of blue-greens, chiefly *Anabaena circinalis, Clathrocystis aeruginosa* and *Coelosphaerium Kuetzigianum,* appeared. At the same time the water had changed from neutral through slightly alkaline to 5 cc. alkalinity during the same time, and on up to 7 cc. alkalinity a month later, indicating the complete absence of free CO_2. This condition remained for over a month, or until September 12, when the rains and cloudy days restored neutrality and reduced the amount of oxygen in solution, partly by the presence of decaying organic matter.

From this time on to middle or late December the amount of oxygen decreased and the CO_2 increased, the alkalinity disappearing and the water showing a large amount of free carbon-dioxide. This does not mean that these are mutual opposites acting like a mechanical couple. The increase in one is not always proportional to the decrease in the other, and vice versa, though in general that is true. There may be either an increase or a decrease in the amount of oxygen without any change in the alkalinity or amount of CO_2, and vice versa; but in general, the conditions which favor the increase of one cause a decrease in the other; in other words, it seems evident that the water is rendered neutral and later alkaline by the withdrawal first of free CO_2 and then of the half-bound CO_2 from the bicarbonates by the rapid growth and active photosynthesis of plankton algæ and shore aquatics which excrete an excess of oxygen. If the water is not disturbed by wind this may go on to supersaturation, which was really the case July 20 to 30, August 21 and September 12. Saturation for that temperature, 27° C., according to the saturation tables of Roscoe and Lunt, is only 5.6 cc. per liter, while my figures for these dates show 7 cc., or 130%, of saturation. Thus while we should expect a decrease in both oxygen and carbon-dioxide with rise of temperature we find, instead, an increase of oxygen, due evidently to plant growth and activity, which diminishes the carbon-dioxide far below the saturation capacity or absorption coefficient.

It will be further noticed that these fluctuations correlate

with cloudiness and sunshine. The first figure, low, with 3.3 cc. of oxygen, June 23, is after rain and cloudy weather, while the high figure, 7.34 cc., July 30, is after a long space of clear weather and the appearance of the "water-bloom," which seems to have been the important agent in producing the increased amount of oxygen.

The lowest figure shown for oxygen, August 25, is after two days of cloudy weather, though the accompanying fall in temperature would increase the water's capacity. Again, on August 28, we have combined rain, cloudiness and wind. The wind diminishes the oxygen content by stirring the water and making supersaturation impossible. September 5, these conditions are augmented by a heavy rain (2.5 inches), which reduced the oxygen figure to 3.44 cc. The heavy rain left the lagoon so clouded as to interfere with photosynthesis, and the organic matter carried in by surface drainage, including some sewage, would greatly reduce the oxygen. It is worthy of note that the decrease was not so great in the upper basin, or Fountain Pool, which was less affected by surface drainage (figures not shown in the table).

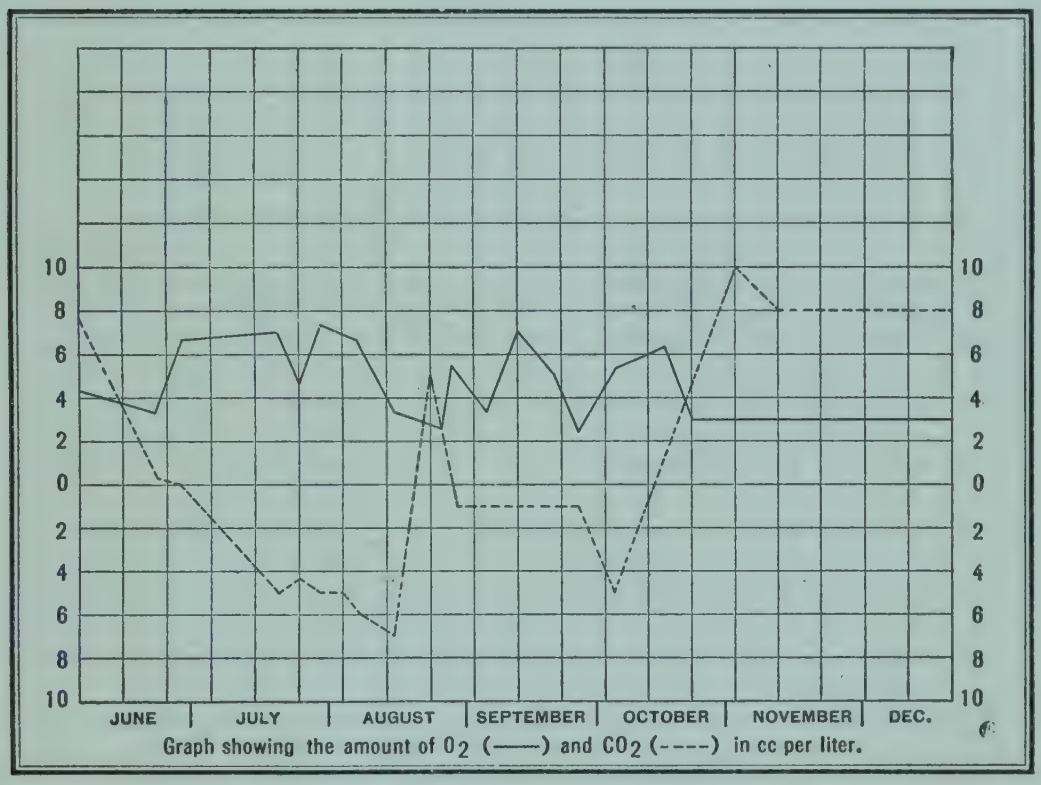
At this time, also, the "bloom" sank out of sight for a time, apparently on account of the cooling of the water, or possibly on account of a change in the specific gravity of the organisms composing the "bloom," so that photosynthesis probably was almost nil in the water darkened by the large amount of matter in suspension. The same conditions were repeated September 26, when the oxygen figure reached its minimum of 2.32 cc. These data, small in number, selected from a much larger number, are presented, not on account of their agreement with any particular theory, but because they are representative. Those not given are omitted simply because they are not significant.

One thing might be misleading in the column on cloudiness, as long lists of clear days have been omitted during which there was no change. This brings a disproportionate number of cloudy days into too close a sequence.

DATE	Temperature Centigrade	Condition of Sky	Oxygen cc. per Liter	Per cent of Saturation	CO_2 cc. per Liter. or Alk. (—)	REMARKS, LOCATIONS, ETC.
June 5 ...	29°	Clear	4.600	84	7.7	In tap water.
June 23 ...	29°	Cloudy	3.360	61	Trace	Lagoon at bridge, air very cool.
June 28 ...	26°	Clear	6.55	116	Slightly Alkaline	After rains.
July 20 ...	26°	Clear	7.10	125	—5.00	Fountain pool.
July 25 ...	27°	Clear	4.53	81	Alkaline	Lagoon at bridge.
July 30 ...	27°	Clear	7.34	131	Alkaline	"Bloom" appeared.
Aug. 7 ...	27°	Clear	6.50	116	—5.00	Fountain pool.
Aug. 15 ...	26°	Clear	3.55	61	—6.00	Smoky, hazy.
Aug. 21 ...	26°	Cloudy	3.08	54	—7.00	Lagoon at bridge after two days of cloudy weather.
Aug. 25 ...	22°	Cloudy	2.60	43	+5.00	
Aug. 28 ...	22°	Cloudy	5.54	92	Alkaline	Shower, windy, 2½ inches rain fell previous night; water over dam.
Sept. 5...	24°	Cloudy	3.44	58	Alkaline	
Sept. 12...	27°	Clear	7.37	131	Alkaline	Sultry day.
Sept. 21...	Air 18° Water20°	Cloudy	5.53	89	Neut.	
Sept. 26...	22°	Cloudy	2.32	39	Neut.	Water muddy after heavy rains.
Oct. 2....	20°	Very Cldy.	5.52	88	Neut.	After 2 days' rain
Oct. 14....	22°	Clear	6.193	102	5.00	
Nov. 21 ...	Air 13° Water 4°	Clear	2.92	32	5.00	Clear and frosty.
Dec. 1 ...	Air 10° Water 3°	Clear	3.00	33	10.00	First ice on lagoon.
Dec. 18 ...	Air 0° Water 0°	Clear	3.00	31	10.00	Thick sheet of ice.
Dec. 27 ...	Air 2° Water 0°	Cloudy	3.50	38	8.00	
Jan. 20....	Air 4° Water 0°	Clear, like April	3.76	41	8.00	Samples from under thick ice.
Feb. 15 ...	Air 12° Water 4°	Hazy	4.18	47	4.25	Thaw after long cold spell, thick ice.

The above figures and the interpretation of them agrees, in the main, with those of Morren ('41) already mentioned. His were given for every day for the entire year. It is hoped that an extension of the above data may serve to confirm the interpretations and conclusions already reached. The later dates are less significant. The "bloom" disappeared with the frost of early November, and there was a decrease in the oxygen content of the water, reaching a minimum again November 21, when the lagoon was almost clear of vegetation. This, with the autumn rains bringing in drainage water, kept the oxygen figure down, while the alkalinity gives place to neutrality for a time, to be followed by an increasing amount of free CO_2, reaching a maximum in December. The

GRAPH I.

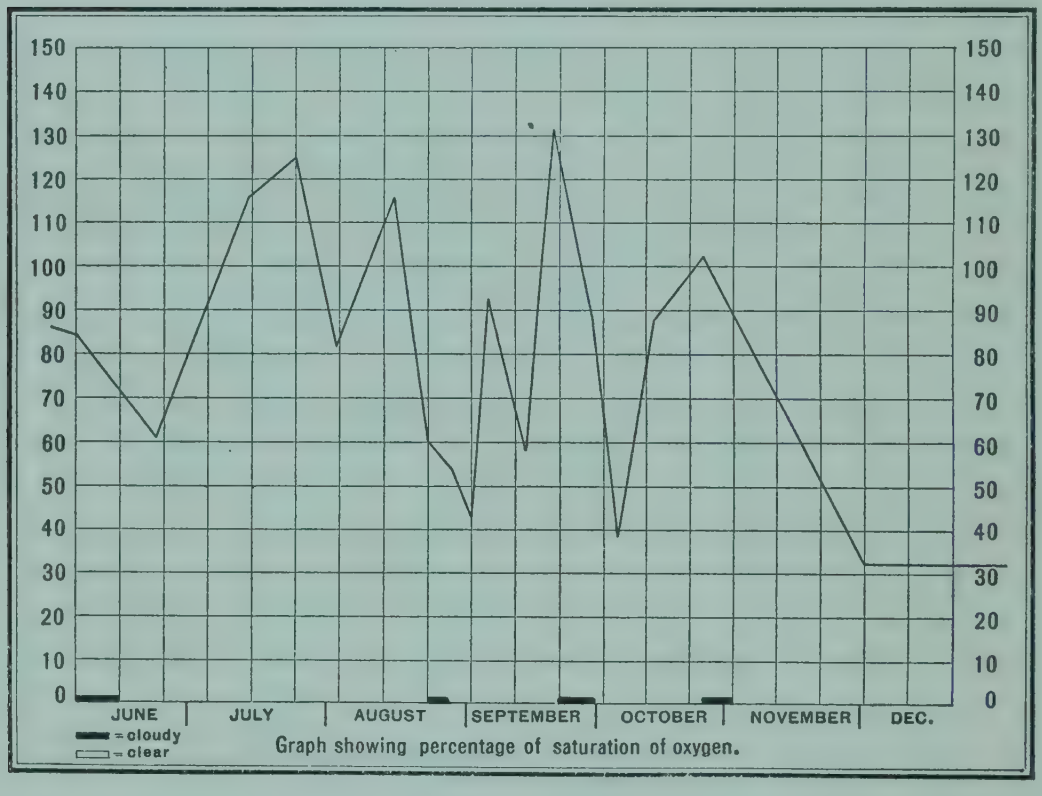


decay of organic matter washed from the dead leaves of a late autumn and the gradually decaying green algæ, together with the decay of the blue-greens of the water-bloom, would materially increase the amount of CO_2 and consume the

oxygen produced by the fall growth of diatoms and grass-greens. After the thick ice sheet was formed about mid-December there was but little change. The oxygen and carbon-dioxide figures both seek the medium and remain there quite constantly. There is but little live vegetation under the ice, and what is there cannot be very active. The same must be true of any animal life that may be present.

The column showing the per cent of saturation and oxygen shows a more striking contrast than the column of cc., which strongly indicates that it must be due to biological rather than to meteorological influences. This is more strikingly shown in the graph showing per cent. of saturation.

GRAPH II.



EXPERIMENTATION IN LABORATORY— GENERAL.

In attempting to arrange a set of experiments under control conditions to show on a small scale the effect of an increase or decrease of the two gases, oxygen and CO_2, a plan was hit

upon of driving these gases, as generated, through a series of flasks partly filled with tap water or pond water, and containing the algæ most available or most likely to respond.

Accordingly, small Erlenmeyer flasks were fitted with two-holed stoppers. Into one hole was a leading-in tube of glass reaching to the bottom. From the other hole was a leading-out tube of glass going to another flask like the first, and reaching nearly to the bottom of the same; and so on through the series. In the same manner the gas driven in at one end could, with little pressure, be forced through the entire series and leave them all saturated and filled with the gas.

Into each flask was put 50 cc. of tap water and a small amount of selected alga. Each flask contained only one species, although there was no attempt at pure cultures nor to rigidly exclude all others.

The generator was connected and the gas passed in to as near saturation as possible; then it was left in diffuse light at ordinary room temperatures, 18° to 20° C. They were left so for about a week when they were again charged with gas. There were two series; one to be treated with oxygen and the other with carbon-dioxide. A third series for control was not to be treated with either.

In order to secure purity of gases and prevent any poisonous or ill effect from that source, a wash-bottle was placed in series between the generator and the culture flasks. The wash-bottle for the CO_2 generator contained a solution of sodium bicarbonate to arrest any HCl gas that might escape from the generator, in which hydrochloric acid and lumps of marble were used. The gas was tested until it gave no precipitate with $AgNO_3$. Preliminary trials showed that this was necessary, as some HCl came over when the wash-bottle was not used, as shown by the above test and the blackening of the plants in the first flasks.

For the oxygen generator the wash-bottle contained only water, as there was no volatile substance nor anything injurious except a little NaOH and a compound of copper in the "oxone," a sodium peroxide compound used for the generation of the oxygen.

The generators were so regulated that the gas came off in a moderately slow stream of bubbles and continued long enough to insure complete saturation. The end flask contained nothing but water and served to prevent the entrance of air or atmospheric gases.

November 14, the following were used and placed in the order named: *Elodea, Pithophora, Cladophora, Spirogyra* and *Anabaena.*

November 21, *Elodea* in oxygen seemed to be dead, while that in the CO_2 seemed brighter than the check. All were renewed and continued as before.

November 26, *Cladophora* in CO_2 was dead and disintegrated.

Cladophora in O_2 was much like check; others unchanged. The above results gave little promise of any conclusions further than to indicate the possibility of continuing an experiment on this plan and that different plants showed a difference in response and endurance. But other factors would likely enter into such treatment, as light, temperature and dissolved salts.

December 7, new material was arranged and continued to December 24, when the conditions were as follows:—

Condition Dec. 24	Large Hydrodictyon	Small Hydrodictyon	Cladophora	Stigeoclonium	Pithophora	Elodea
Check	Bright	Spoiled	Unchanged	Dead	Green	Dead
In CO_2 ...	Fair	Improved	"	"	"	Green
In O_2.....	Good	Very Bright	"	"	"	Nearly Dead

A large amount of *Elodea* was kept in jars in various conditions as to light, etc., and it all did well, so the material was not at fault. *Stigeoclonium* did not do well under any conditions.

Not much change occurred in any of the cultures, except that *Clathrocystis* (from which important results were expected) soon died in all the series as it had done under various kinds of treatment throughout the summer. One large jar, after the excessively hot weather was over, kept in

moderate light and temperature (about 20° C.), lived for over a month, but died, eventually, without any apparent cause.

In the above table it will be seen that *Hydrodictyon* in oxygen became much brighter than the check or that in CO_2. This was true of all the others in oxygen, though in less degree. This fact, though needing further confirmation, agrees with that of Senn ('99), obtained with *Cœlastrum*. He placed cultures of *Cœlastrum reticulatum* in culture solution (0.2% Knop's solution) in Erlenmeyer flasks, one set being supplied with oxygen and the other with carbon-dioxide somewhat as my cultures were. He placed some in the dark and some in diffuse light. After six days the flasks were opened and investigated. He says: "Schon dem blossen Auge boten die Sauerstoffculturen einen ganz anderen Anblick als diejenigen mit Kohlensäuere. Während man bei den letzteren mit Mühe am Boden des Fläschens einen grünen Schimmer entdecken konnte, zeigten die Sauerstoff-culturen einen deutlichen, prächtig frisch grünen Boden-überzug. Unter dem Mikroskop stellte es sich nun heraus dass die mit Sauerstoff cultivirten Algen zahlreiche einzelne, prächtig grüne aussehende Zellen gebildet hatten; dieses frisch grüne Aussehen hatte seinen Grund darin, dass die Zellen ihre Stärke völlig verathmet hatten."

Still further, and, perhaps, more remarkable, he produced bright green chlorophyll in yellow resting cells of the same plant in ten days, by means of a culture solution saturated with oxygen, while similar cultures supplied with CO_2, instead of oxygen, produced only a reddish-yellow color. These cultures, with CO_2, were placed in the light. Of those containing oxygen (four in all) two were placed in the light and the other two were left in the dark. Oxygen cultures in distilled water remained entirely red, showing that culture solution (food) was necessary for the formation of chlorophyll.

With *Cœlastrum microporum* he was less successful, especially in cultures exhausted with the air-pump. The cultures with oxygen showed at the end of the week almost

exclusively bright green cells, scarcely colored by iodine. Those left in the dark had increased little or none, were pale green and turned dark brown when treated with iodine. The first series of CO_2 cultures nearly all died on account of the strong concentration of the gas used. A second experiment with a weaker concentration of CO_2, after five days, showed chiefly cœnobia with spherical cells which colored intense brown with iodine. In general, the species *C. microporum* was less resistant and more likely to perish. It was also more apt to form single free cells and less so to form cœnobia than in the case of *Cœlastrum reticulatum.*

Among my cultures was a flask containing, among other things, *Cœlastrum microporum* in Knop's solution, which had been standing quietly on a laboratory table for six months. On examination it was found to contain many cœnobia of eight, sixteen, and some of thirty-two cells; some of these were beginning to form daughter-cells, or young cœnobia. No such large colonies were to be found when the original material was taken from the pond in September. Although the culture fluid was shallow in the flask, about 1 cm. deep, and the flask plugged only with cotton, the conditions were essentially those of stagnant water, and agree closely with those employed by Senn ('99) in some of his work, though it would require duplicates and extensions to justify any final conclusions.

Some *Cladophora* placed in a glass jar with a loose glass lid and nearly filled with tap water (stagnant condition) showed a much more branched condition after a number of weeks than the original specimen taken from flowing water, or any of the specimens found in free open water, although the water, light and other conditions, aside from aëration, were the same. The new cells, moreover, were shorter and thicker than in the older parts of the original specimen, and the cells had broad, thickened, or inflated ends, that showed a branched appearance or an abnormal tendency toward branching. In the older cells the length is usually ten or more times the diameter; but in these newer cells, and especially the end ones, the length is scarcely three or four times the diameter.

Fritsch ('09), in noting the seasonal changes of *Cladophora* in Abbot's Pool, near Bristol, England, describes the inflated type as characteristic of winter, and the much-branched type as characteristic of winter, late spring and summer; so it is a little difficult to interpret this or relate it to any reported conditions.

A similar tendency was shown by one culture of *Hydrodictyon.* In this the cells had all acquired a zigzag form, with inflated, club-shaped ends. They soon developed spores, or gametes, and disintegrated, with no further development. This was in an open jar of tap water which had evaporated to about one-eighth of its original volume.

Freund ('08), in an extensive article on the influence of environment on vegetative reproduction, says: "More probable, though less clear, is the influence of the oxygen content of water. The assertion that zoospore production can be induced merely by treatment with oxygen, as stated by Waltz, has been disproved by Klebs for *Vaucheria repens* and *V. clavata.* Simply by increasing the oxygen content in the surrounding medium, so far as our present knowledge goes, or may allow us to judge, no alga can be aroused to zoospore formation. On the other hand, has it not been accomplished by the removal of oxygen? Klebs made a study of *Vaucheria* and *Hydrodictyon,* which indicates that many algæ *(Vaucheria repens, V. clavata, Hydrodictyon,* and *Oedogonium diplandrum; Stigeoclonium tenue, Draparnaldia* and *Ulothrix zonata)* can be induced to form zoospores if they be removed from flowing water to stagnant water, which indicates that reduction of oxygen in the medium is, in this case, the regulating responsible factor. The possibility of the influence of a reduction in oxygen is not excluded when algæ like *Botrydium* and *Vaucheria,* growing in moist air, produce zoospores by moistening with water. Naturally, in this case, other influences come in, such as the absorption of water, etc., which were not previously distinguishable."

None of the cultures, so far as I could discover, showed any tendency to zoospore formation in response to the stimulus of oxygen or CO_2. The tendency toward periodic spore

production seems to be too deep-seated in heredity to be easily overthrown by any artificial external stimulus. The observations of Fritsch ('07), Benecke ('08), Copeland ('09) and Danforth ('10), lend great weight to this idea, as do also the studies of Williams ('05) on the Dictyotaceæ. However, Benecke and Fritsch concede that if we could fully imitate the conditions found in nature we might get the same results regardless of season.

EXPERIMENTATION IN LABORATORY—SPECIAL.

In order to test more fully what I had noticed, as to the water becoming alkaline by the growth of algæ, I arranged several series of cultures with and without carbonates of various kinds.

November 24. Test-tubes were filled with distilled water and boiled to drive out any CO_2 that might be present; then they were stoppered and allowed to cool. When cool, small quantities of the following plants were put in: *Hydrodictyon, Oedogonium, Elodea* and *Spirogyra.* The tubes were stoppered as tightly as possible and placed near an east window. In another set of test-tubes with distilled water as in the others, instead of boiling, a little powdered calcium carbonate was used, which was intended to absorb all the free carbon-dioxide. These were supplied with plants from the same source as the others, stoppered in the same manner and placed alongside the others, near a window.

December 24. At the end of a month the *Oedogonium* and *Spirogyra* in the tubes of boiled water were dead and black, attacked by moulds and beginning to putrefy. The *Hydrodictyon,* though still green and apparently alive, showed no oxygen bubbles or other sign of activity, like that of the check material. The *Elodea,* likewise, was green, but dormant. All the tubes containing lime carbonate were bright and active, as shown by an abundance of oxygen bubbles. Since all the tubes were tightly stoppered to exclude the air, the only source of CO_2 for photosynthesis was that of the bicarbonates, which showed that the plants named could utilize the CO_2 from this source.

The above-named plants had been kept in stock in open jars or flasks supplied with tap water, which contains considerable lime in solution and is slightly alkaline to phenolphthalein. This usually loses its color when plants are first introduced, but becomes deep red again after a day or two in bright sunlight or passing CO_2 through the water. A period of darkness had the same effect of destroying or withdrawing the color. But the color returned again within a few hours of bright sunlight, and the process was repeated with the alternation of darkness and light.

This I attributed to the action of photosynthesis during the day, withdrawing CO_2 from the water, thus changing the solution from a weakly acid one to a neutral and alkaline one, causing the red color; and respiration by night or the balance of respiration over photosynthesis on cloudy days restoring the lost CO_2 and again rendering it neutral, or acid and colorless.

This I believed at the time, to be an original discovery and interpretation of my own, but on investigation found that, among a number of others, Hassak ('88) had given the same interpretation, backed by proofs at considerable length. To this, however, Loew ('93) found objections, and claimed that the coloring was due, not to a normal carbonate of lime, as explained by Hassak, but to the excretion of a colloidal compound of calcium, which is alkaline to phenolphthalein. Hassak, in proof of his stand-point, states that sodium bicarbonate gave the same results as calcium bicarbonate, and he believed that other carbonates would do the same if he only had the opportunity to try them.

Hassak used 0.1% and 0.2% solutions in his experiments. Accordingly I prepared 0.1% and 0.2% solutions of sodium bicarbonate, using melted snow, as it was necessary for the experiment that the water be free only from carbonates.

January 9. Into these were put *Elodea, Pithophora, Cladophora, Oedogonium, Hydrodictyon* and *Spirogyra.* Another set of blanks was used as a control, containing only water and plants, and another with nothing but water. To each tube were added three drops of phenolphthalein (1%

in 50% alcohol). The tubes were boiled, cooled, and the plants introduced. They were then tightly stoppered and placed in the light of a south window.

February 6. After nearly a month the blanks remained colorless. This showed that the alkalinity did not come from the excretions of the plants themselves, as it had been claimed by Loew. All the tubes containing both plants and sodium bicarbonate turned deep red on bright days, and faded during the night or cloudy days, which were numerous enough at that time. *Spirogyra,* alone, gave little or no color, owing, apparently, to its poor condition. The intensity of the color varied with the plants in the order named, *Elodea* giving the deepest, and *Spirogyra* the faintest. There seemed to be little, if any, difference between the 0.1% and 0.2% solutions. The results were in all essential respects similar to those in the solutions of lime-carbonate.

Cultures prepared in the same manner, but using potassium bicarbonate, gave even more decided results. This is all the more significant since cultures with potassium nitrate, instead of carbonate, failed to give any color, as Loew claimed, but soon died, instead, and became infested with moulds. In so far this indicated that the important thing is the carbonate in the production of alkalinity.

February 2 to March 2. A similar set of experiments, using ammonium-carbonate, resulted only in the death of the plants. This was likely due to the toxic effect of the ammonium which, according to the experiments of Livingston ('05), is five times as toxic as the above cations in the same concentration, and also Andrews ('05) showed that hairs of *Tradescantia virginica* were killed if left longer than one minute in a one per cent solution of ammonium-carbonate, and were affected adversely in a 0.5% or 0.25% solution of the same.

Another set of cultures like the above, but containing manganese carbonate, although run for nearly a month, gave no color. This is probably due to the low degree of solubility and dissociation of this compound.

One of the strongest arguments against the theory of Loew

is the fact that the first time this color reaction was noticed by me was in a small flask of pond water left in a south window during the very bright and very hot days of the summer of 1911. The color appeared faintly at first and increased from day to day until the cause was searched out.

At first the flask seemed to contain nothing but the water, no plant material being visible; but closer investigation showed that the change in color was due to a few microscopic algæ, chiefly Desmids, *Pediastrum* and the like, which, as before mentioned, have little tolerance for calcareous waters, and do not, as a rule, have lime deposited on their surfaces. These algæ developed mostly as a result of multiplication in the flask.

INCREASED AND REDUCED CO_2 AND O_2 PRESSURE.

In order to test the conduct of higher pressures of CO_2 upon the dissolved carbonates and contained algæ, the following experiments were prepared:—

A. February 10. A half-pint milk bottle was half-filled with distilled water; three small crystals of $KHCO_3$ were added and three drops of phenolphthalein, as in former experiments; this gave no color. Into this were put about 5 grams of *Pithophora*. The bottle was then stoppered with a rubber stopper, attached to a suction pump and exhausted to one-half vacuum, then refilled to atmospheric pressure with CO_2 from the attached generator. The jar was then closed air-tight and placed in the light of a south window. In a few days the color appeared, indicating photosynthesis and the reduction of bicarbonate to normal carbonate. The color faded during the night and cloudy days, reappeared on bright days, more especially when in the bright sunlight, just as the previous cases with open vessels and tap water.

B. A control jar like "A," except that tap water alone was used, without $KHCO_3$. This went through the usual color changes.

February 24. Jar "A" was opened and a lighted match applied. The flame flared up more brightly, showing that

the CO_2 had been absorbed and that oxygen had been thrown off instead. During the interval masses of bubbles appeared in this jar just as in the control and in previous experiments, evidently bubbles of oxygen, as shown by the lighted match, and showing that vigorous photosynthesis had been going on in the jar, making use of the excess CO_2.

March 1. This jar, "B," was again exhausted and refilled as before, causing the color to disappear rapidly as the CO_2 was absorbed, thus changing the normal carbonate to bicarbonate. After a few days the color reappeared, bubbles formed, showing continued photosynthesis as before.

March 14. A lighted match indicated, as before, that the CO_2 had been absorbed and replaced by oxygen.

As further control for comparison, a jar half-full of tap water, which was alkaline to phenolphthalein, was half exhausted and refilled to atmospheric pressure with CO_2, which quickly dispelled the color just as when the plants were present. But the color did not reappear when placed in sunlight, as it did when the plants were present. This seemed to leave no further doubt that the alkalinity and color changes were due to the photosynthetic activity of the plants, thus appropriating the CO_2.

February 27. Another experiment like the above was set up in which *Hydrodictyon* was substituted for *Pithophora,* using only tap water, and the same amount of CO_2 as before. The color faded in about 20 minutes and did not reappear again when placed, like the other, in the south window for over two weeks.

March 16. The *Hydrodictyon* seemed colorless and dead.

March 16. An attempt to revive with oxygen was a failure. Apparently this concentration of CO_2 is too great for *Hydrodictyon,* which is not so resistant as *Pithophora.*

Schützenberger ('73), on the basis of some work with *Elodea,* states that for water plants 5% to 10% of complete saturation with CO_2 is the optimum. I have not found any account of work done on algæ with high per cents of carbon-dioxide.

APPARATUS AND MATERIALS.

In all experiments in which measured amounts of gases were employed, or definite pressures of gases were desired, a Bunsen suction pump with manometer was used, and gas generators were attached in the manner described by Wesbrook ('05), or somewhat similar to that described and used by Chapin ('02) in his studies on the influence of CO_2 on plant growth.

The method of collecting and determining the amounts of oxygen in water was that described and used by Whipple and Parker ('02) in their studies. The method is commonly known as Winkler's method. It is more fully described by Birge and Juday ('11).

Another method, based upon the reducing power of ferrous sulphate, was also used as a check and as an alternative, many determinations being made by both methods.

Samples were usually taken and determined in duplicates, and, in case of doubt, were many times repeated. The purest of chemicals obtainable were used throughout.

SUMMARY.

1. There is an intimate and mutual relation between the algæ and submerged aquatics in a body of water and the gases dissolved in that water. They fluctuate together.

2. Air, or its constituents, oxygen and CO_2, are as essential to water plants as water is to land plants, and equally difficult to secure.

Warm and stagnant water is poorer in these essentials than colder water gently agitated by wind or currents.

4. Currents are especially beneficial to attached plants by renewing or removing these gases.

5. Some species demand more aëration than others. Some species are more tolerant of stagnant waters than others.

6. Filamentous forms with large cells and thin outer walls are best adapted to stagnant waters. Such forms predominate in warm, tropical fresh waters, which are poorly aërated.

The photosynthesis of rapidly-growing algæ and aquatic

plants in a body of water may diminish or deplete the supply of CO_2 and increase the oxygen content beyond saturation.

8. In the absence of free CO_2 the plants may utilize the half-bound CO_2 of the dissolved bicarbonates, chiefly those of calcium and magnesium.

9. The process of photosynthesis may be so vigorous as to exhaust the half-bound CO_2 and render the water alkaline. By respiration and absorption of CO_2 from the air more bicarbonates may be formed. This serves as a mechanism for the conservation of CO_2.

10. Waters rich in lime-carbonates are also rich in vegetation. Bog waters, containing humic acids, and, consequently, poor in carbonates of lime, are known to be poor in vegetation.

11. Stagnant water, on account of the large amount of CO_2 and the small amount of oxygen, favors the formation of colonies and filaments rather than of free individual cells.

12. Colonies and filamentous forms may be produced artificially with some plants, by increasing the amount of CO_2 or diminishing the amount of oxygen in the culture solutions.

13. Narrow, much-branched filaments are adapted to and produced by poorly aërated waters.

14. Aëration, or abundance of oxygen, apparently favors the formation of chlorophyll; and algæ are brighter green when well aërated.

15. The periodicity of spore formation is not readily influenced by aëration or gas content of the water. It seems to be more a matter of heredity.

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